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Non-Dietary Human Exposure and Risk Assessment

EDITED BY

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Foreword

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Before agreeing to publish a book, the proposed table of contents is reviewed for appropriate and comprehensive coverage and for interest to the audience. Some papers may be excluded to better focus the book; others may be added to provide comprehensiveness. When appropriate, overview or introductory chapters are added. Drafts of chapters are peer-reviewed prior to final acceptance or rejection, and manuscripts are prepared in camera-ready format.

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Preface

The Protection of Subjects in Human Research rule by the USEPA, including the establishment of the Human Studies Review Board (HSRB), has resulted in changes to both study design and study evaluation processes, particularly with respect to ethical considerations. *Non-Dietary Human Exposure and Risk Assessment* is a compilation of the presentations given in a symposium of the same name at the 238th ACS National Meeting in Washington D.C. The purpose of the symposium was to provide a forum for scientists from industry, academia, and government to share investigative methods used to generate data for use in non-dietary human risk assessments and to share methodology for performing and evaluating those assessments.

This compilation is intended to provide the reader with a concise overview of the current status of both the scientific and regulatory aspects of non-dietary human exposure and risk assessment as applied to pesticides. It is the hope of the editors that it will also be the starting point for discussions leading to the further refinement of study and risk assessment design, data evaluation, and regulatory harmonization.

Three major areas are covered in this symposium edition. The first area is regulatory issues including the development of the Protection of Subjects in Human Research rule and the HSRB, statistical procedures involved in designing human exposure studies, handling of the data generated in those studies, and quality assurance processes related to worker exposure studies. The second area, study design, includes processes for the identification and recruitment of volunteers for human exposure studies, overviews of several studies that have been recently performed, the development of procedures for evaluating the resulting data by Regulatory Agencies, and efforts towards international cooperation in the generation and use of exposure data. The final area, methodology, includes examples of the development of methods for the analysis of samples generated in non-dietary human exposure studies with particular emphasis on the use of hyphenated techniques and the development of a model for determining greenhouse exposures that is currently being used in Europe.

The editors would like to thank all of the contributing authors for sharing their expertise in this developing area at the confluence of research and regulation. Special thanks are due to the Agricultural Handlers Exposure Task Force (AHETF) and the USEPA Office of Pesticide Programs (OPP) for providing leadership in this emerging area.

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Chapter 1

Non-Dietary Human Exposure and Risk Assessment: Regulatory Issues

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The assessment of the non-dietary exposure and resultant risk potential to pesticides has been an integral part of the regulatory process in the United States, Canada, and the European Union (EU) for decades. Non-dietary exposure assessments require an understanding of the dermal, inhalation, and incidential oral routes of exposure. This area involves exposures that not only result from the occupational handling of pesticide products, but also re-entry into treated fields, non-occupational exposures to home and school applied pesticides, and potential exposure from applications to individuals not involved in the application, typically referred to as bystander exposure. As we enter the second decade of the 21st century there are significant changes occurring regarding regulatory issues involving non-dietary exposures and risk assessments. These issues include the growing use of the risk assessment process in jurisdictions outside of North America and the EU, the development of newer exposure data and databases, and a re-evaluation of the risk assessment process.

Introduction

The assessment of the non-dietary exposure and the resultant risk potential to pesticides has been an integral part of the regulatory process in the United States, Canada, and the European Union (EU) for decades. Non-dietary exposure assessments require an understanding of the dermal, inhalation, and incidential oral routes of exposure. This area involves exposures that not only result from the occupational handling of pesticide products, but also field worker re-entry into treated fields, non-occupational exposures to home and school applied pesticides, and potential exposure from applications to individuals not involved in the application, typically referred to as bystander exposure.

As we enter the second decade of the 21st century there are significant changes occurring regarding regulatory issues involving non-dietary exposures and risk assessments. New non-dietary exposure studies are being conducted to update the data previously used to conduct exposure assessments. With the development of the new data there are also efforts to develop new databases to handle the data. The conduct of non-dietary exposure studies for submission to the U.S. Environmental Protection Agency (EPA) must be conducted under new guidelines intended to protect the human subjects of these studies. The evaluation of residential exposure to pesticide residues is being re-evaluated by the EPA with particular emphasis on improving the understanding of childrens' behavioral patterns. Exposure that results from the off-site deposition of agricultural pesticide applications is gaining attention and guidance on the development of assessment methods is now ongoing in North America and the EU. Finally, there are new concerns that may require the conduct of non-dietary exposure and risk assessments that have not previously been conducted. In December 2009 the EPA issued a new policy regarding the conduct of risk assessments not governed by the Federal Food, Drug, and Cosmetic Act (*1*). In that policy notice the EPA announced its intention to address non-dietary exposures to teenage workers and to young children taken into agricultural fields by their working parents.

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New Data Development

Extensive efforts are currently underway in North America and Europe to update the non-dietary exposure data that have previously been relied upon for regulatory risk assessment purposes. In North America the Agricultural Handlers Exposure Task Force (AHETF) has been developing mixer/loader and applicator data to update the agricultural exposure data that currently reside in the Pesticide Handlers Exposure Database (PHED). The PHED database has been used by both the EPA and Canada's Pest Management Regulatory Agency (PMRA) since the 1990's. PHED has also been used by regulatory authorities in other jurisdictions such as Australia. The AHETF studies are being conducted under harmonized study designs and current agricultural practices. A similar effort is also underway by the Antimicrobial Exposure Assessment Task Force II (AEATF) to conduct occupational exposure studies involving the use of antimicrobial pesticide products. Both of these North American task forces have cooperated with each other regarding study designs and methodology issues. In the EU similar efforts by the agricultural chemical industry are ongoing to develop occupational exposure data to update the agricultural exposure data currently relied upon in the European Union. An example of this effort is the development of greenhouse handler exposure model under the auspices of the European Crop Protection Association (ECPA). Exposure data from numerous studies conducted in southern European greenhouses are intended to address data gaps identified in the current United Kingdom Predictive Operator Exposure Model (UK POEM), the German BBA model, and EUROPOEM.

The AHETF, AEATF, and ECPA have been jointly developing a new database designed to handle the new data being developed and to replace the existing databases or models being used by regulatory authorities in the US, Canada, and EU. This new database is called the Agricultural Handlers Exposure Database or AHED™. While the programming will be the same, each task force will enter its own data into the the program creating versions specific to North American agricultural pesticide products, North American antimicrobial products, and European agricultural pesticide products. The programming for the AHED™ database will be made available to anyone interested in the database for potential use with the appropriate data in other regulatory jurisdictions such as Brazil or Latin America.

On 6 February 2006, the EPA issued a final rule for the protection of subjects in human research (*2*). The rule, among other requirements, strengthened existing protections for adult subjects in research for pesticides by individuals other than EPA who intend to submit the results of the research to EPA. The Protection of Subjects in Human Research rule governs the conduct of all non-dietary human exposure studies conducted for submission to EPA. One of the requirements for third parties conducting such research is submission of protocols for human research studies to the EPA prior to study initiation. The EPA must conclude that the proposed study design is scientifically valid and will be conducted under accepted ethical standards if the data from the study are to be submitted to EPA. EPA must also submit its evaluation and all supporting documentation to the Human Studies Review Board (HSRB), a federal advisory committee established by the final rule,for comment and advice. In a similar fashion the completed studies and supporting information must also be submitted to EPA to determine if the data are scientifically valid and the study was conducted in an ethically acceptable manner. EPA must submit its evaluation of the completed study and all supporting information to the HSRB for comment and advice. The implementation of this rule has significantly affected the conduct of non-dietary exposure studies performed for EPA submission. The effort to comply with the rule has been difficult at times and a learning process for third parties, the EPA, and the HSRB. There does appear to be a consensus that implementation of the rule has had a positive impact on the AHETF and AEATF studies subject to the rule.

Residential Exposure Assessment

Residential exposure to pesticides incorporates many uses of pesticide products in addition to the lawn, garden, and indoor uses that are normally thought of. The use of pesticide products on pets, as insect repellants, or impregnated into materials such as clothing or furniture are also included in this category. Guidelines for assessing the non-dietary exposure resulting from the residential uses of pesticides was first issued by EPA in 1997 with modifications

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in 1999. Since the issuance of the first Residential Exposure Standard Operating Procedures (SOPs) new exposure data have been developed by the Outdoor Residential Exposure Task Force (ORETF) and other third parties, by EPA's Office of Research and Development (ORD), and academia. EPA has evaluated these new data and is currently in the process of revising the SOPs (*3*). These draft technical guidelines were presented to the FIFRA Science Advisory Panel (SAP) in October 2009. The proposed new SOPs provide additional and, in some cases, more robust data and advanced assessment capabilities, such as stochastic and probabilistic tools. In most cases, the exposure scenarios and basic algorithms have remained the same as the current SOPs with changes made only to the algorithm inputs using more recent data sources. However, some new scenarios have been added to this set of SOPs reflecting new products and uses and some existing scenarios have modified exposure algorithms. In addition, where possible, distributions for the algorithm inputs are provided for use in probabilistic models. It is interesting to note that the proposed SOPs often do not yet provide recommended point estimates for the conduct of deterministic risk assessments. The recommended point estimates are intended to reflect an as yet undetermined percentile of exposure. This appears to reflect an ongoing policy development within EPA regarding the appropriate percentile of the exposure distribution to be used in the residential risk assessment process.

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Bystander Exposure

Non-dietary exposure to agricultural drift is an area undergoing significant regulatory changes as the second decade approaches. The term "drift" is intended to include any airborne movement of pesticides away from the target site during and/or after application. This can include airborne movement of pesticide droplets, pesticide powders, and volatilized vapor-phase pesticides. Bystander exposure itself can involve either a short-term and intermittent exposure from a individual passing near an application or it can involve the exposure to deposited residues or vapors at a site adjacent to an agricultural establishment such as a residence or school. Bystander exposure has been addressed in the EU under Council Directive 91/414/EEC for some time. With some limited exceptions quantitative bystander exposure assessments have typically not been conducted for EPA or PMRA regulatory purposes with some exceptions.

The methodology used to assess bystander exposure in the United Kingdom was challenged in 2008 in Georgina Downs and Secretary of State for Environment, Food, and Rural Affairs (*4*). In his ruling Judge Collins determined among other things that, *The result of this judgment is that the defendant must think again and reconsider what needs to be done. It is not for me to specify any particular action he needs to take. He must take steps to produce an adequate assessment of the risks to residents. In addition, he must carefully reconsider whether the existing conditions of use are adequate. The need to inform residents of intended spraying and of the composition of the pesticides to be used is I think clear. Voluntary action is not achieving this. Equally, I think there is a very strong case for a buffer zone, such as incidentally already exists to avoid*

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spraying too close to watercourses in order to minimise the risk of pesticides entering groundwater. The UK government appealed the ruling by Judge Collins and in July 2009 the three judge appeallate panel allowed the appeal by the Secretary of State for Environment, Food, and Rural Affairs. During this time period a cooperative effort by members of the European agricultural chemical industry and German regulatory agencies proposed guidelines for the assessment of bystander exposure (*5*). The approach described by this effort was to satisfy the requirements for the protection of bystander and resident health under the German regulatory legislation and to contribute to the harmonization of the assessment procedures for the Europe-wide bystander and resident exposure assessment scenarios under Council Directive 91/414/EEC.

The development of guidelines for assessing bystander exposure in the US appears likely in the near future. The EPA has begun to address the development of an approach for assessing inhalation exposure resulting from the field volitalization of conventional pesticides. Presented to the FIFRA SAP in December 2009, the EPA has proposed a two-tiered approach when appropriate data are not available and an approach for when appropriate data are available. During the same time period the EPA issued two Federal Register Notices regarding spray drift. The first notice seeks comments on draft pesticide drift labeling intended by EPA to provide clearer direction and consistency across States with regard to enforcement of pesticide drift labeling statements. The second notice involved a petition to EPA prepared by Farmworker Justice and Earthjustice to protect children from pesticide drift. One of the key elements of the petition is that EPA develop a method to evaluate the exposure of children to pesticide drift. The EPA appears to be moving in the direction of developing guidance on the assessment of bystander exposure based on its proposed policy changes for revised risk assessment methods (*1*). In that proposed policy the EPA has committed to assess the risks posed to bystanders near agricultural fields that may be exposed to pesticides via volitalization and/or drift.

Future Regulatory Issues

Two additional areas regarding non-dietary human exposure assessments were raised in the December 2009 EPA revised risk assessment methodology proposal. The EPA is currently evaluating the issue of occupational exposure to children age 12 to 17 years who work in agriculture. Based on the proposed policy changes the EPA currently believes that the occupational exposure potential to this cohort is sufficiently similar to adults to not warrant a separate exposure assessment. The EPA is also preparing to assess the non-dietary exposure to young children taken into agricultural fields by their working parents. The proposed methodology appears likely to be broadly similar to current post application exposure assessment methods with the development of contact and exposure factors specific for children and the development of incidental oral exposure methods.

Regulatory ssues involving non-dietary human exposure and risk assessments are rapidly evolving and it is hoped that the chapters in this book will provide the reader with greater insight into these issues.

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Chapter 2

Establishment of the Human Studies Review Board (HSRB)

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The establishment of the Human Studies Review Board (HSRB) follows a long history of ethical concerns. While extreme examples associated with the last World War come to mind, there are numerous activities in the United States that increased pressure for further human protection. A profound lapse in ethical behavior is the Tuskegee Syphilis study. This study, sponsored by the US Public Health Service, spanned 40 years from 1932 and recruited 399 African American males from Alabama for a study of "bad blood." In fact, the purpose of the study was to follow these men as they developed late stage syphilis. While effective treatment for syphilis was not available at the beginning, penicillin became available in the 1940's. This treatment was intentionally withheld from study participants. It was only after the study was exposed on national media and public outrage ensued that the study terminated. As a direct consequence, the National Research Act of 1974 was passed increasing protections for human subjects. Institutional Review Boards were established a year later by the Department of Health, Education and Welfare. The 1979 Belmont Declaration further defined the ethical considerations governing medical studies. In 1988 the Interagency Human Subjects Coordinating Committee increased protections. The 1991 Common Rule (40 CFR Part 26) established ethical guidelines for government agencies. As important as the Tuskegee study was in alerting the country to the need for ethical reform, the Food Quality Protection Act (FQPA) of 1996 was the final catalyst for HSRB establishment. A provision of

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the FQPA was the institution of an additional 10-fold safety factor to protect sensitive populations such as infants and toddlers. The practical effect of this change in uncertainty factors (UFs) was that the total UF could now be 1000 or as high as 3000 where previously it was 100 or 300. As the UF increased, the ability of pesticide registrants to register products decreased. The hazard identification and dose response were essentially constant input factors in risk assessments; a lower reference dose (e.g., NOAEL ÷ 1000 UF) could increase the chance that the RfD would be exceeded. Faced with this barrier, a number of human dosing studies to establish human NOAELs were submitted to EPA. In effect, the animal to human 10-fold safety factor could be eliminated, reverting the combined UF to 100. The submission of these human studies was perceived by NGOs as an attempt by registrants to skirt the increased safety restrictions of FQPA. Intense controversy ensued. The EPA, in response, asked a joint Scientific Advisory Board / FIFRA Science Advisory Panel to review the issue of human studies. It also asked the National Research Council of the National Academy of Sciences to weigh in. Both reviews concluded (with some dissent) that human studies conducted to the highest ethical and scientific standards are appropriate for the Agency to use in pesticide registration decisions. EPA said it would consider human studies on a case by case basis in October 2001 but reversed itself in December in a press release stating it will not consider or rely on any such human studies in its regulatory decision making, whether previously or newly submitted. The Agency was then taken to Court by Industry since their pronouncement was a rule promulgated without formal rulemaking. The US Court of Appeals found for the Industry plaintiffs. Faced with the requirement for establishing clear guidelines for the use of human data, the Agency sought to apply guidelines embodied in the Common Rule to studies conducted by Third Parties (e.g., Industry). The Appropriations Act of 2006 directed the Agency to establish an Independent Human Studies Review Board and the Agency issued a Proposed Rule for the Protection for Subjects in Human Research on September 12, 2005. The Final Rule, published January 26, 2006, established the HSRB. Its effective date was April 7, 2006.

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Introduction

The practical effect of the establishment of the Human Studies Review Board (HSRB) has been to curtail the initiation of intentional human dosing studies by the agrochemical companies. The path to the establishment of the HSRB was influenced by past unethical behavior, government's attempt to prevent further unethical medical transgressions, and the United States Environmental Protections Agency's decisions that reconciled two opposing views: those of Non Governmental Organization (NGOs) that were firmly opposed to human testing and the Agrochemical Industry that considered human testing a legitimate and valuable addition to pesticide safety data.

The Environmental Protection Agency Regulates Pesticide Registrations

The Fungicide, Insecticide and Rodenticide Act (FIFRA) was first established in 1947 under the US Department of Agriculture (*1*). It was revised in 1972 at which time it was transferred to the newly established Environmental Protection Agency (EPA, Agency) (*2*). Collectively, FIFRA and associated Agency guidance says that the EPA Administrator must consider all reliable relevant data when conducting risk assessments required for determining whether an agrochemical may be registered for use.

Risk Assessment at the Agency

The bulk of toxicology data submitted to the Agency are derived from non-clinical laboratory studies. Typically, these include acute toxicity, subchronic studies, developmental and reproductive studies, mutagenicity studies, and chronic studies. From these data no observed adverse effect levels (NOAELs) are established that determine whether or not a proposed agrochemcial has sufficiently low risk, based on estimated exposure. A key component of this risk characterization is the application of Uncertainty Factors (UFs) to the NOAELs to calculate reference doses (RfDs). The RfD is that dose to which persons can be exposed without unacceptable risk of adverse effects.

Traditionally, the UF used in toxicology risk assessments has been 100. This originally was based partly on tradition and was noted as a combination of two UFs: a 10-fold UF in moving from animals to man and a 10-fold UF to account for the variations encountered within the human population.

The Food Quality Protection Act of 1996 (FQPA) mandated that EPA add an additional 10-fold UF to ensure protection for vulnerable subpopulations such as infants and toddlers (*3*). This additional UF could be reduced to 1 (i.e., eliminated) if the database was essentially complete and data showed that young animals were not more sensitive to the adverse effects of the agrochemicals compared to adults.

The practical effect of FQPA was to raise the bar for registrations. By example if the critical NOAEL were 10 mg/kg/day, the application of a 100-fold UF would create an RfD of 0.1 mg/kg/day. With FQPA, the additional 10-fold UF would create an RfD of 0.01 mg/kg/day. In some cases, by example if the NOAEL were

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based on subchronic versus chronic studies, a 3-fold UF would also be applied. This would result in a collective UF of 3000 and an RfD of 0.003 mg/kg/day.

Magnitude of the Residue studies (MORs) would allow an estimation of dietary intake based on agrochemical usage. For occupational workers, that is Mixer, Loader and Applicators along with Reentry workers, scenario-based exposure studies would allow an estimate of dermal and inhalation exposure.

For both dietary and occupational risk, it is a simple matter to calculate the estimated exposure against the derived RfD. Dermal exposure would also factor in the absorption rate, estimated from non-clinical studies, typically in rats. Once systemic exposure was calculated, if it exceeded the RfD, mitigation measures would normally be put in place to lower exposure such that the RfD was no longer exceeded.

A summary of this process is shown below, where UF_{AtoH} is the animal to human UF, UF_H is the human UF and UF_{FQPA} is the FQPA UF.

$$\mathbf{NOAEL \div (10UF_{AtoH} \times 10UF_{H} \times 10UF_{FQPA}) = RfD}$$

Use of Human Data Will Permit a 100-Fold UF

In the above equation, the total UF is 1000. The use of human data eliminates the need for one of the 10-fold UFs, effectively increasing the RfD. While agrochemical registrants had submitted human data to the Agency prior to passage FQPA, an increased number of human studies followed its passage.

Submission of Human Studies Triggers Intense Controversy

FQPA was intended to increase protections against adverse effects due to pesticide exposure. Some Non Governmental Organizations (NGOs) characterized the submission of these human studies as an attempt by the agrochemical industry to subvert the purpose of FQPA. They (e.g., the Environmental Working Group, EWG, and the National Resource Defense Fund, NRDC) were opposed to the use of human data and urged EPA to not allow these data to be used in their decision making process.

EPA Seeks Guidance on Whether to Use Human Data

In an effort to resolve this problem, EPA asked two expert bodies to consider the use of human studies in evaluating agrochemicals for registration. The first was the joint Science Advisory Board - FIFRA Scientific Advisory Panel committee: Data from Testing of Human Subjects Subcommittee (DTHSS), which issued their report in 2000: *Comments on the Use of Data from Testing Human Subjects* (*4*). The second was the National Academy of Sciences (NAS) that issued a report in

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2004: *Intentional Human Dosing Studies for EPA Regulatory Purposes: Scientific and Ethical Issues* (*5*).

Both the DTHSS and NAS concluded that human studies that were conducted to high ethical and scientific standards were appropriate for consideration by EPA. There were, however, some members of the DTHSS who disagreed with this conclusion and issued a minority dissent.

EPA Responds to the Expert Committee Recommendations

In October 2001 EPA noted it would consider human studies on a case-by-case basis. Two months later, however, the Agency issued a press release stating that, *...the Agency will not consider or rely on any such human studies in its regulatory decision-making, whether previously or newly submitted.*

This reversal was in effect rule making without following the usual rule making procedures. As a result, the Agrochemical Industry took the Agency to Court. The United States Court of Appeals, District of Columbia Circuit found, in 2003, for the Plaintiff, CropLife America, against the EPA (*6*). The Court's decision required the Agency to develop clear guidance as to how human studies would be used in the regulatory process.

A summary of some of these milestones is noted in Table I.

Ethics in Medicine

In the Introduction key developments leading up to the establishment of the HSRB were noted. Underlying the concern for the use of human studies is the issue of medical ethics.

Medical Ethics Has a Long History

One could cite the Hippocratic oath as the basis for medical ethics, paraphrased: *above all else do no harm*. Table II notes some medical studies or procedures that are now considered unethical. These range from intentionally infecting people with pathogens to irradiating patients without their consent to the more outrageous 'medical experiments' conducted during World War II.

Much as our society needs a police force to maintain societal order, it is apparent from the ease at which unethical procedures are employed, that oversight regulations need be in place to maintain ethical order.

The Tuskegee Syphilis Study

This one study, sponsored by the United States Public Health Service, is both the 'Poster Child' of unethical medical studies as well as the touchstone that initiated a cascade of actions leading to the establishment of the HSRB (*7–11*).

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Basis for the Tuskegee Syphilis Study

Physicians believed that the development of syphilis proceeded differently in Caucasians compared to African Americans. They were interested in the progression of this disease in African Americans and recruited nearly 400 men in Macon County, Alabama. The nature of the study was not clearly conveyed to these recruits. They were told it was about "bad blood." Since it was the first time that many of the participants actually had medical attention, there was an incentive to continue participation. At the time of the study start (1932) there was no effective cure for syphilis. Treatments of the time included mercury, bismuth and arsenic. Aspirin replaced even these treatments for the Tuskegee patients.

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Table I. Milestones Leading to the Establishment of the HSRB, see also (*12*)

Year	*Milestone*	*Comment*
1932	Tuskegee Syphilis Study initiated (*10*, *11*)	Public Health Service sponsored investigation of the progression of syphilis in African American males
1947	Nuremburg Code (*13*)	Guidance in response to WWII medical "experiments"
1947	FIFRA Enacted (*1*)	Pesticide regulations under USDA
1964	The Helsinki Declaration (*14*)	World Medical Association guidance
1972	FIFRA Amended (*2*)	Pesticide regulations under EPA
1972	The Tuskegee Syphilis Study terminated (*8*, *15*)	Public concern forces the US PHS to cancel this study
1974	National Research Act (*16*)	An important step in improving the protection of human subjects, triggered in large part by the revelations of the Tuskegee Syphilis Study
1979	The Belmont Report (*17*)	National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research
1986	Office of Science and Technology Policy (OSTP) proposes Model for Federal Policy for Protection of Human Subjects (*18*)	
1988	Interagency Human Subjects Coordinating Committee revises Protection of Human Subjects (*19*)	Informed Consent; Inter Review Boards (IRBs)
1991	The Common Rule (*20*)	Guidance for the protection of human subjects for federal agencies

Continued on next page.

Table I. (Continued). Milestones Leading to the Establishment of the HSRB, see also (*12*)

Year	*Milestone*	*Comment*
1996	The Food Quality Protection Act (FQPA) (*3*)	An additional 10-fold Uncertainty Factor may be applied for risk assessment
1998	Environmental Working Group publishes "The English Patients" (*21*)	A critique of the use of human data since FQPA was passed
1998	EPA Suspends reliance on human data while it conducts an evaluation of its policy	
2000	DTHSS Report (*4*)	Studies conducted to the highest ethical and scientific standards may be used by EPA for risk assessments (minority dissent included)
2001	EPA will evaluated human data on a case by case basis (October), referenced in (*7*)	
2001	EPA will not consider human data in their pesticide regulations (December), referenced in (*22*)	"Rulemaking" issued in the form of a press release
2003	US Court of Appeals finds for the agrochemical industry against EPA (*6*)	The Court affirms that EPA must develop clear guidelines for the use of human studies
2004	NAS Report (*5*)	Studies conducted to the highest ethical and scientific standards may be used by EPA for risk assessments
2005	HHS revisions to the Common Rule (*23*)	Subparts A, B, C, D
2005	Appropriations Act of 2006 (*24*)	Congress specifies EPA cannot use human data until a HSRB is established
2005	Proposed Rule, September 25th (*25*)	Application of the Common Rule to Third Party studies. Occupational Exposure studies are excluded
2006	Final Rule, effective April 7th (*26*)	HSRB is established. Occupational Exposure studies are now included in the definition of intentional dosing studies.
2006	HSRB convenes, April 4th (*27*)	HSRB meets 3-4 times/year

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Table II. Samples of Studies Conducted in the Past (*28*)

Year	*Study*
1895	Patient is infected with gonorrhea
1900	Doctors infect Filipino prisoners with plague
1911	Rockefeller Institute physician injects patients with syphilis
1915	US PHS officer produces pellagra in inmates
1932	Start of the Tuskegee Syphilis study
1941	Vanderbilt University prenatal women given radioactive iron
1942	Army and Navy doctors infect 400 prisoners with malaria (to help the war effort)
1939-1945	World War II medical “experiments”
1944	Plutonium injected in soldiers as part of the Manhattan Project
1950	US Army releases clouds of cadmium over six US and Canadian cities
1956	US Army releases mosquitoes infected with yellow fever and dengue in Savannah, GA
1963	Dr. Southam injects cancer cells into prisoners and senile African American patients
1967	CIA places chemicals in drinking water of FDA headquarters
1995	Participant in MIT study of airborne pollutants dies
1999	FDA approved gene therapy clinical trial induces the death of one participant

Questionable Ethics Morphs into Intentional Unethical Conduct

Medicine advanced and an effective treatment for syphilis, penicillin, was discovered in the 1940’s. It was at this stage that the physicians running the Tuskegee study made a conscious decision to withhold treatment. This was, in effect, a death sentence to many of the participants as they suffered and died from tertiary syphilis. Clinical signs from terminal syphilis are grotesque, often leading to premature death.

Throughout the remainder of the study participants were cajoled with messages indicating that they must come in for important treatment. The treatment, in many cases, was another spinal tap.

A Former PHS Employee Points Out the Unethical Nature of the Syphilis Study

Peter Buxton tried to get the study terminated. The Public Health Service rebuffed his suggestions. After he was ignored, he spoke with an Associated Press reporter who wrote a story that was published in the Washington Star on July

25, 1972. The initial response of the PHS, however, was to say these men were volunteers and were always happy to see the doctors. One State health official commented that someone was trying to make a mountain out of a molehill.

When the national press picked up on the story, public response caused the PHS to reverse its position and the study was finally shut down in 1972. In 1997 President Clinton formally apologized on behalf of the government and the Public Health Service.

The Tuskegee Study Paves the Way for HSRB

Tuskegee Triggers the Passage of the National Research Act of 1974

Two key elements were introduced by the National Research Act: 1) the establishment of informed consent and 2) the establishment of Institutional Review Boards (IRBs) (*16*).

The Act considered risk-benefit criteria, how human subjects were selected, and the nature and definition of informed consent.

Previous Codes Laid the Foundation for the National Research Act

The Nuremberg Code (13)

There were ten directives to the 1947 Nuremberg Code: 1) Voluntary consent is essential; 2) The experiment should yield fruitful results; 3) The experiment should be based on the results of animal experimentation and a knowledge of the natural history of the disease; 4) The experiment should avoid unnecessary physical and mental suffering; 5) No experiment should be conducted where there is a prior reason to believe that death or disabling injury will occur; 6) The degree of risk should never exceed that determined by the humanitarian importance of the problem; 7) Proper preparations should be made and adequate facilities provided; 8) Scientifically qualified persons should conduct the study; and, 10) The scientist in charge must be prepared to terminate the experiment at any stage.

The Declaration of Helsinki (14)

There are six Basic Principles and eight Operational Principles to the 1964 Declaration of Helsinki.

Basic Principles include: 1) Respect for the individual; 2) Right to make informed decisions; 3) Investigators duty is solely to the patient or volunteer; 4) The subject's welfare must always take precedence over the interests of science and society; 5) The increased vulnerability of special groups calls for special vigilance; and 6) When the subject is incompetent or a minor, allowance should be considered for surrogate consent. This last Principle has been disputed.

Operational Principles include: 1) Research should be based on knowledge of the science; 2) Conduct assessments of the risks and benefits; 3) Reasonable likelihood for benefit to the populations studied; 4) Conducted by trained

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investigators; 5) Use approved protocols for ethics that have continued oversight by an independent committee such as an IRB; 6) Discontinue study if original considerations no longer are satisfied; 7) Information should be publicly available; and 8) Interests of the subjects continue after the study.

Subsequent Codes Advance Ethics in Human Studies

The Belmont Report (17)

The National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research met at the Smithsonian Institution's Belmont Conference Center and issued their report April 18, 1979: Ethical Principles and Guidelines for the Protection of Human Subjects of Research.

The Belmont Report stresses three fundamental ethical principles when human subjects are used for research: 1) Respect for persons: protecting the autonomy of all people and treating them with courtesy and respect and allowing for informed consent; 2) Beneficence: maximizing benefits for the research project while minimizing risks to the research subjects; and, 3) Justice: ensuring reasonable, non-exploitative, and well-considered procedures are administered fairly (the fair distribution of costs and benefits to potential research participants).

The Principles of the Belmont Report remain the basis for Health and Human Services protection regulations and is an important reference for IRBs.

The Common Rule (20)

In 1991 40 CFR Part 26 was published in the Federal Register. This established the guidelines for protection of human subjects in studies conducted by the United States Government. It is known as the Common Rule. Over fifteen Agencies subscribe to its guidance and rules. The Office for Human Research Protections (OHRP) in the Department of Human Services was instrumental in the development of the Common Rule. In 2005 there were five subparts: A) Basic HHS Policy for Protection of Human Research Subjects; B) Additional Protections for Pregnant Women, Human Fetuses and Neonates Involved in Research; C) Additional Protections Pertaining to Biomedical and Behavioral Research Involving Prisoners as Subjects; and D) Additional Protections for Children Involved as Subjects in Research.

HSRB Is Established

The confluence of four factors worked to establish the HSRB: 1) Expert Committees concluded that human studies conducted to high levels of ethics and science should be used by the Agency; 2) Efforts by NGOs to limit the use of such data by EPA; 3) The Court's directive that EPA must set clear guidance on how data will be handled; and, 4) The Agency's need to resolve this controversial subject.

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The resolution of this problem was to extend the provisions of the Common Rule to Third Parties; that is, human studies conducted by registrants (First Party studies are conducted by EPA; Second Party studies are sponsored by EPA).

The Appropriations Act of 2006 (*24*)

This Act (Public Law 109-54, signed August 2, 2005) addressed the issue of intentional dosing human toxicity studies for pesticides and directed the Agency to establish an independent Human Subjects Review Board to review such studies.

Section 201 of this Act states: "None of the funds made available by this Act may be used by the Administrator of the Environmental Protection Agency to accept, consider or rely on third-party intentional dosing human toxicity studies for pesticides, or to conduct intentional dosing human toxicity studies for pesticides until the Administrator issues a final rulemaking on this subject. The Administrator shall allow for a period of not less than 90 days for public comment on the Agency's proposed rule before issuing a final rule. Such rule shall not permit the use of pregnant women, infants or children as subjects; shall be consistent with the principles proposed in the 2004 report of the National Academy of Sciences on intentional human dosing and the principles of the Nuremberg Code with respect to human experimentation; and shall establish an independent Human Subjects Review Board. The final rule shall be issued no later than 180-days after enactment of this Act."

The Proposed Rule (*25*)

The Proposed Rule: Protections for Subjects in Human Research was issued September 12, 2005. Comments were collected and reviewed for issuance of the Final Rule.

EPA Acknowledges the Controversy over Human Study Data

Under the heading of Societal concern over Ethically Deficient Human Research, the Agency noted: "For over 7 years EPA has been at the center of an intense debate about the acceptability of certain intentional dosing human studies for pesticides, and about what to do with human studies which are ethically deficient. In this debate some have argued that EPA should disassociate itself entirely from ethically problematic research behavior by refusing to consider the resulting data in its regulatory decisions. Those who hold this view interpret Agency reliance on an ethically flawed study as an endorsement of the investigators' behavior, and as encouragement to others to engage in similarly problematic research. They also argue that EPA's reliance on ethically deficient human data could directly benefit the wrong-doer. For example, if EPA based a regulatory decision on a human study that shows humans to be less sensitive than animals, the result might be a less stringent regulatory measure that would be advantageous to the company that conducted the study. If the key study was ethically deficient, then the company could benefit from its misconduct.

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"On the other hand, data from human research has contributed enormously to scientific understanding of the risks posed by every kind of environmental substance. Recognizing the importance of such knowledge to EPA's past regulatory actions, some argue that the Agency should take all relevant and scientifically sound information--not excluding ethically deficient human data--into account in its regulatory decision-making. They argue that any ethical deficiencies are the fault of the researchers, not of EPA. They further argue that by relying on scientifically valid and relevant data from an ethically deficient study EPA does no additional harm to the subjects of the research, and EPA's refusal to rely on such data could do nothing to benefit the subjects of the research. Moreover, they assert that while the Agency cannot undo what has already happened, EPA can clearly express its disapproval of past unethical conduct. They note that to replicate scientifically sound but ethically flawed human studies may not be ethical, no matter how carefully such replicate research might be conducted, since any increment of risk to potential subjects would not be justified by anticipated new generalizable knowledge. Holders of this view also stress the importance of strengthening protections for volunteers who participate in future studies, while taking advantage of all that can be learned from past research to benefit society."

EPA References Past Codes and Extends the Common Rule to Third Parties

The Agency noted the Belmont Report, the Common Rule and the World Medical Association's Declaration of Helsinki. The Common Rule, which previously applied to federal agencies would now be extended to apply to Third Party Research; that is, research by companies not supported by EPA.

The Proposed Rule Excludes Agricultural Occupational Exposure Studies

The proposed text defining what constitutes intentional exposure of humans notes, "a study of an environmental substance in which the exposure to the substance experienced by a human subject participating in the study would not have occurred but for the human subject's participation in the study."

"Examples of studies that do not meet this definition included 'monitored agricultural workers (such as professional fruit thinners or harvesters or other workers) who perform their usual work in areas that have been treated with pesticides at rates and using methods registered and approved by EPA. While they are participating in the research these workers' urine and blood may be collected for analysis to evaluate biological responses, or they may wear patches attached to their clothing that are collected at the end of the shift for analysis to measure exposure."

The Proposed Rule Includes the Human Studies Review Board

The Agency noted, “EPA proposes to require prior submission of protocols and related information for proposed third-party human research covered by the rule. This rule as proposed would apply to the same range of research to which EPA’s Common Rule would be extended--i.e., all intentional dosing human studies intended for submission to EPA under the pesticide laws. EPA also proposes to establish a Human Studies Review Board to provide an additional scientific and ethical peer review for such research. Finally, the Agency proposes to require that submitted reports of covered third-party studies include detailed documentation of the ethical conduct of the studies.”

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The Final Rule (*26*)

The Final Rule was issued January 26, 2006 and became effective on April 7, 2006. With its implementation the Human Studies Review Board was established.

The Final Rule Reflects the Proposed Rule with Some Exceptions

The important change that occurred between the Proposed and Final Rules was the decision to include occupational exposure studies where they previously were excluded. The Agency noted, it ...“has decided that the types of research captured by the definition of ‘research involving intentional exposure of a human subject’ is broader than suggested by the preamble to the proposal. Although the text of the definition remains the same, EPA thinks it is important to clarify that the term covers any research on a substance, unless the subjects of the research retain complete control over whether, when, and how they are exposed to the substance. Thus, if a researcher decides a particular compound will be studied in the research and determines the manner in which subjects will be exposed, the research falls within the scope of ‘research involving Intentional exposure.’”

HSRB Initiates Reviews of Protocols and Studies

The first meeting of the HSRB took place April 4, 2006 (*29*). It has met three to four times per year since. Issues tackled include completed intentional dosing of human studies with pesticides, protocols for studying efficacy of insect repellants, and occupational exposure studies. This review touches only briefly on the effect that the HSRB has had on pesticide research. The reviews of the Agricultural Handler Exposure Task Force (AHETF) protocols, however, reflect some of these effects.

The initial review of the AHETF protocol met with a number of questions from the Board. Subsequent submissions to the HSRB made incremental improvements in the Board’s judgment of the ethics and science of the proposed work. Intense effort on the part of AHETF in consultation with the Agency has allowed the task force to again begin fieldwork. Often, however, with continued HSRB review, additional issues arise that must be addressed.

There are two practical effects of the establishment of the HSRB: 1) the costs of conducting intentional human dosing studies has increased due to longer time lines and increased supporting documents that need to be generated; and, 2) in the view of the Agency and others the transparency of the risk assessment process has been significantly improved. On whole, the combinations of competing interests (cost of doing business versus more transparent regulatory actions) favor the continued function of the HSRB.

Since the establishment of the HSRB, there have been no protocols submitted for review whose objective is the study of organophosphates and their NOAEL for cholinesterase inhibition in humans.

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Conclusion

The Human Studies Review Board embodies society's need for ethical and scientific oversight when humans are involved in experiments. There is a history of Codes and regulations that both preceded and was essential for its establishment. Practically, it solves the contentious issue that faced the US EPA: how human data should be used in pesticide regulation.

The benefits that the HSRB brings to society must be balanced by recognition of its costs. The process of developing data from intentional human testing has slowed (i.e., they are more costly both in terms of time and money); studies that might have provided valuable data to our understanding of human toxicology may not now be conducted; and, the Agency's ability to conduct occupational risk assessments using high quality data, has been delayed while the Agricultural Handler Exposure Task Force 're-tools' to meet the needs of HSRB.

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Chapter 3

Impact of Human Studies Review Board on Agricultural Worker Exposure Studies

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In February, 2006, the Final Rule strengthening existing protections for human volunteers in research studies was enacted. Along with extending new protections to subjects involved in pesticide research, the law created a new, independent Human Studies Review Board (HSRB) to advise the EPA on the ethical and scientific issues arising in such research. The process for gaining approval to conduct studies involving human volunteers has changed radically. Standard protocols that have been used for decades to conduct occupational exposure monitoring studies have been transformed as a result of input from HSRB. Many changes provide additional protections and rights for the volunteers. Additionally, the HSRB has had an impact on the science aspects of study protocols, including enhancement of the study objective, review and discussion of existing exposure data, statistical considerations, and fundamental changes in test subject identification and recruitment.

Background

The protection of human subjects for research purposes has evolved since laws were initially established in the United States in 1906 (*1*). One of the most memberable steps towards improving standards for the ethical treatment of human volunteers was the Nuremberg code which was established in 1947. Since then additional safeguards including the Declaration of Helsinki in 1964 and the Belmont Report in 1979 have been put into place to ensure that human subjects are informed, respected, and treated ethically. In 1981 the Department of Health

In Non-Dietary Human Exposure and Risk Assessment; Krolski, M., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2010.

and Human Services and the Food and Drug Administration issued regulations based on the Belmont Report. In 1991 the core of these regulations (45 CFR, Part 46, Part A) referred to as the "Common Rule" were formally adopted by several other branches of the US government, including the EPA, who either conducted or funded research involving human volunteers. However, this rule did not apply to third parties, including other government entities and private industry.

It was not until February 6, 2006, that the Common Rule was extended to include third party intentional dosing research designed to support pesticide registrations. This new rule, referred to as the "Final Rule" contains input from the National Academy of Sciences, was funded in part by the Appropriations Act of 2006, and took over five years to develop. In addition to improving the existing ethical standards covered by the Common Rule, the Final Rule strengthens the protections of specific sensitive sub-populations by prohibiting new research involving intentional exposure of pregnant or nursing women or children intended for submission to EPA under the pesticide laws. Additionally, it established an independent Human Studies Review Board (HSRB) designed to peer review both protocols for new research and completed third-party intentional dosing research intended to be used to support EPA registrations of pesticides.

The HSRB is a Federal advisory committee that reports to the EPA administrator through the EPA's Science Advisor (*2*). The purpose of this board is to provide advice, information, and recommendations to the EPA on the science and ethics of proposed and completed research involving human subjects as well as recommendations on how to strengthen the agency's program for protection of human subjects. Typically composed of 16 members, the advisory board includes experts in relevant scientific or technical disciplines such as bioethics, statistics, human health risk assessment, and human toxicology. Board members are expected to attend and participate at all meetings and participate in preparing the reports and recommendations. Board members may serve for a term of three years, utilizing a system of staggered terms of appointments (*2*).

HSRB meetings are held approximately four times a year at three month intervals and are open to the public. The general format includes introductory presentations and evaluations by EPA followed by clarification questions from the panel before discussion of key questions that have been provided in advance to the panel. Comments from the public are solicited and short presentations during the public input period are allowed. Meetings typically occur over a period of three to four days. Documents, including protocols, relevant Standard Operating Procedures, and other supporting material, for proposed research must be submitted to EPA at least 75 days before the scheduled HSRB meeting (*3*). Completed study reports must also be submitted at least 75 days before the scheduled HSRB meeting (*3*).

The role of the HSRB is to review and evaluate intentional dosing studies conducted with human subjects intended to support pesticides registrations. The definition of "intentional dosing" in this context means that if it were not for participation in the study, the individual would not have handled or been exposed to the particular chemical or product (*4*); another term for intentional dosing is scripted. Examples of such studies include: the measurement of skin irritation or skin sensitization; studies designed to evaluate the absorption and

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excretion of a chemical; sensory (taste or odor) perception studies; efficacy of insect repellants; exposure measurements to products if the product would not have been used on the particular day of the study. The monitoring of agricultural workers handling pesticides typically falls under this last example and is thus considered an intentional dosing study under the Final Rule. Scripting in this case would include specifying quantities of pesticide used, equipment used, length of workday, number of tank loads, and the specific active ingredient used. Even if the agricultural worker has used the particular EPA-registered chemical many times in the past and will be monitored while using it according to labeled directions, the study is likely to be considered a scripted study and subject to HSRB review.

Studies can also be classified as observational if certain criteria are met. An observational study is a study where the activities or environment has not been modified for the purpose of the study (*4*). Data collection, whether video, notes, or samples, does not require any alteration to a person's typical daily routine. Examples of observational studies include activity pattern documentation, behavioral analysis, surveys, and exposure monitoring of people who would be exposed whether or not they participated in the research (*4*). Generally agricultural worker exposure studies have not been done as observational studies because usually a specific pesticide is under investigation and a minimum amount must be handled to assure detectable residues on the sampling media.

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Changes in Agricultural Worker Exposure Studies

Up until the implementation of the Final Rule, the majority of agricultural chemical companies and their contractors in the United States followed similar procedures for the design and implementation of GLP-compliant worker exposure monitoring studies. In general these studies followed the EPA OPPTS Series 875 guidance (*5*) with respect to sampling methodology, number of subjects, and number of geographic locations. For studies involving the monitoring of workers during groundboom or airblast operations, the guidelines require a minimum of five replicates at a minimum of each of three locations. The locations for the study were typically based on where the crop or chemical of interest was normally grown or used. The pool of volunteers was based on the desired crop type, number of acres needed, geographic region, and application equipment requirements. Recruitment was an informal process generally handled by local field contractors with expertise in agricultural research. Once the study volunteers were identified, a well documented informed consent process was implemented, usually by the Study Director or the Principle Field Investigator. Standard features of these worker exposure studies intended to support pesticide registrations included informed consent forms, confidentiality of worker identification, and disclosure of potential adverse effects, product labels and MSDS.

The entire process for human volunteer identification, selection, and recruitment changed radically following input from the HSRB during the two year period from June, 2006 to June, 2008. A large number of other procedures ranging from test subject literacy considerations through justification of the scientific need to conduct the study were also implemented as a direct result of input from the HSRB. All of these changes stem from HSRB reviews of protocols submitted by the Agricultural Handlers Exposure Task Force (AHETF) starting in June, 2006, when the initial five agricultural exposure monitoring protocols were reviewed and subsequently rejected (*6*).

Additional Human Protections

Based on the recommendations of the HSRB, there have been a significant number of changes and additions specifically focused on ethics and protection of subjects. Some of these recommendations were already standard practice, while others were being implemented to a lesser degree. The following list provides some examples of the considerations which are now required to be included in worker exposure monitoring protocols.

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1. Institutional Ethics Review – an independent ethics review of the study protocol and informed consent form by an institutional review board is now required for every worker exposure monitoring study. In the past it was common for registrants to obtain an independent ethics review for studies involving atypical activities with pesticide treatments such as turf hand-press or Jazzercise re-entry studies; however many companies did not regularly obtain IRB approval for occupational exposure monitoring studies involving the labeled use of a registered pesticide. This has now changed – all studies involving human subjects must go to an IRB. The submission package that goes to EPA and the HSRB must not only include the IRB approvals, but also the minutes from the IRB meetings; a list of names, degrees, affiliations, and employment of IRB members; records of continuing review activities associated with the research; and all correspondence between the IRB and the investigators.
2. Informed Consent Form – In the past it has been standard to prepare a consent form and for the field investigator to make sure that the participants had read and understood the form. Now the Study Director must also determine whether the test subject understood the consent form. In order to accomplish this, a short comprehension test must be administered by a researcher during the consenting process. This documentation is retained along with the consent form.
3. Spanish-speaking workers – occasionally Spanish-speaking volunteers are used in mixer/loader and applicator or re-entry monitoring studies. In the past Spanish-English translators have been brought to the field to assist with those workers who do not speak English. At the request of the HSRB, the use of Spanish translators is no longer acceptable. Instead, bilingual researchers must be used.
4. Illiterate volunteers – study candidates must speak English or Spanish, but are not required to read. There needs to be a clear procedure to determine whether a person is illiterate or not, and this should be done in a manner that would not cause embarrassment or discomfort to the volunteer. In order to accommodate volunteers who cannot read the

consent form, the form will be read to them by a designated member of the research team.

5. Ethics training – all members of the research team as well as anyone who may have contact with the test subjects must take an on-line ethics training course.
6. Photographs – photographs taken of the test subjects during their participation in the study should not contain distinguishing features such as their faces or tattoos. In the past photographs and video were taken of the subjects while performing their work activities, but typically no effort was taken to conceal the worker's identity.

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New Technical Aspects

Based on the feedback from the HSRB and EPA, there have been a number of general changes and additions to the protocols. The following list provides some examples of what is now included in worker exposure protocols.

1. Number of workers per farm – there is no limit on the number of workers per grower/farm or commercial facility that can be placed in the pool of subjects; however there can be only one worker per grower/farm who participates in the study. In the past multiple workers from one farm or one operation could be used in a monitoring study. This new requirement makes it difficult to monitor more than one worker per day, thus increasing the amount of time needed to complete a study and driving up study costs significantly.
2. Recruitment procedures – local agricultural researchers are no longer used to identify and recruit volunteers. In order to incorporate elements of random selection into the process and to decrease bias, a complex, multi-step procedure is now used to identify, select, and recruit volunteers. This process can involve a significant number of people including a professional recruiting center. As such, the time and resources needed for recruitment have increased significantly. A detailed description of this procedure is in the chapter authored by Victor Cañez (*8*).
3. Justification for data generation – protocols now require a description of the existing exposure data and why additional exposure monitoring data are needed. Although this might seem to be outside the scope of ethical matters, if there is no valid scientific rationale for generating the data, the study is considered to be unethical.
4. Incorporating random elements – rather than being completely purposive in design, protocols should contain as many elements of random selection as possible. This can be implemented in the choice of test site location, crops, and/or application equipment and/or recruitment of test subjects. The protocols should include a description of the rationale and methods for data collection; a description of efforts made to incorporate random elements; and a description, rationale, and justification for the selections. The concept that adding randomness to these studies to remove or

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reduce bias that might otherwise skew the results may be overstated. The purpose of these studies is to generate exposure data for a specific agricultural scenario. The central tendency of those data are then used to calculate daily exposure and are compared to product-specific toxicological data to estimate occupational risks. Exposure data are primarily influenced by interpersonal variability and have been shown to differ by an order of magnitude within the same study. Given the low relative precision of exposure measurements, it is unclear how these arduous extra steps to add randomness to the protocol are actually impacting the final outcome, the range of exposure measurements. The incorporation of these random elements has considerably increased the time and resources needed to plan and initiate a worker exposure study.

5. Societal value of proposed research – a discussion of the societal value of the proposed research is now needed. This is in addition to a discussion of benefits and risks associated with the use of human subjects in the study.

Increased Timeline

The EPA and HSRB review process has effectively doubled the time, from approximately 9 months to approximately 18 months, needed to conduct an exposure monitoring study. Since there are only four HSRB meetings scheduled per year, the choice of the review meeting needs to be coordinated with the timing of the study and allow for approximately one month to receive written feedback from the board. Additionally the paperwork must be submitted to EPA at least 75 days prior to the scheduled HSRB meeting. As such, these new requirements could easily add on an additional eight to twelve months to the process.

The preparation of the submission can take several weeks to several months. There should be only one submission per study, with a table of contents and continuous pagination (*3*). There are four distinct sections as follows:

1. Scientific research: protocol, SOPs
2. Informed consent process: recruitment process, consent form, recruitment flyer, product risk statements including Spanish translations
3. Ethical oversight: IRB approval, minutes from IRB meetings, IRB & registrant correspondence
4. Reference materials: copies of scientific information cited

A five page submission checklist prepared by EPA should also be consulted and included (*3*). For a task force which is dealing with several study protocols to cover one use pattern, this presents an unusual and challenging mission. The June 2008 submission by AHETF was for two airblast applicator studies and contained 9 volumes totaling approximately 2,000 pages (*7*).

The other significant change that has added a considerable amount of time and expense to conducting an exposure monitoring study is the test subject recruitment process. What used to be accomplished in a matter of weeks is now a complicated step-wise procedure that involves a recruitment center, several specialists, and up to several months of work. Identification of sources of the population of interest can begin early in the protocol development process; however, calls to establish the list of prospective participants cannot be made until the protocol has been reviewed and approved by the IRB, EPA, and HSRB (and the California Department of Pesticide Regulation if the study is to be conducted in California).

Increased Costs

The EPA and HSRB review process has also added considerable costs to the conduct of an exposure monitoring study. The process itself – preparing the necessary documents for submission to EPA; interacting with EPA; preparing for and attending the HSRB meeting – has added new costs. The many additions to the protocol, consenting process, random elements, and the elaborate recruitment process have all added expense to the preparation phase of these studies. Another consequence of the HSRB's desire to eliminate bias is that only one worker per farm or commercial spray company can participate in the study. This basically restricts monitoring to one worker per day, increasing the time needed to conduct the field phase of the study which in turn adds to the expense. The AHETF has calculated that the costs to conduct these studies have almost tripled since 2006, from an average of $18,000 per monitoring unit (replicate) to approximately $50,000 per monitoring unit. These costs reflect the added work in preparing for the testing program; new procedures for recruiting workers; a restriction to monitor no more than one worker per location/farm; the inability to monitor mixer/loaders and applicators in the same study; and overall loss of efficiency.

Case Study: HSRB Review of AHETF Airblast Applicator Study

Two of five planned field study protocols for measurement of potential dermal and inhalation exposure during application of liquid pesticides using closed-cab conventional airblast sprayers were reviewed at the June 24-25, 2008 HSRB meeting (*7*). These IRB-approved study protocols along with extensive supporting documentation were submitted to EPA on April 7, 2008 and represented two years of work.

At the HSRB meeting both the science and ethics of the proposed research were evaluated. The board agreed that the existing exposure data for closed-cab airblast application were inadequate and agreed that the planned studies presented minimal risk to the volunteers (*7*). They also accepted the revised statistical-based sampling design of a hybrid purposive sampling with random elements. There were approximately 40 recommendations, most of which were incorporated into the protocols or the SOPs. The remaining recommendations were either incorporated in the protocols submitted for the October, 2008, HSRB meeting or were evaluated for possible inclusion in future studies. Several required no action.

The sampling design contained both purposive and elements of random selection. Key elements include: 1) selection of study crops, study areas, and strata of pounds handled per acre; 2) identification of growers of the target crop in the study areas; 3) random contact of growers; 4) compilation of data from eligible growers willing to cooperate; 5) design of an efficient configuration for monitoring; and 6) recruitment of workers by randomly selection when multiple

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qualified workers are employed by the same grower. This sequence of steps was detailed in several Standard Operating Procedures.

The HSRB written comments were published on September 11, 2008 (*7*). Once the recommendations were addressed, the AHETF was able to move forward with conducting these two studies. The remaining three protocols for this use scenario were reviewed and approved at the October, 2008 HSRB meeting.

Conclusions

The HSRB review process presents a large hurdle for those agricultural chemical companies wanting to conduct studies involving the use of human test subjects. Preparation for this public process is time consuming. The recommendations of the HSRB have resulted in some substantial changes to how agricultural exposure monitoring studies are planned and conducted. The HSRB looks not only at the ethics of the study, but also at the science. Worker exposure monitoring protocols now have an increased emphasis on the protection of workers and must include a justification for the generation of these data. The statistical design, justification for selection of representative crops and locations, characterization of the population of interest, and identifying an unbiased population of test subjects are all important new components to the protocol.

Since the implementation of the Final Rule in February, 2006, there have been relatively few new protocols submitted to support the registration of pesticides. Currently the AHETF has gained approval of nine worker exposure monitoring protocols (June, 2008; October, 2008; and June, 2009). Another industry-sponsored task force, the Antimicrobial Exposure Assessment Exposure Task Force (AEATF), obtained approval for two exposure monitoring protocols in April, 2008. Although several individual registrants have gained approval for insect repellency studies involving human volunteers, to date no individual agricultural chemical company has submitted a protocol for new research.

In order to conduct a study that meets these new standards, companies need to allocate additional time and resources. Studies need to be planned at least a year in advance. In addition to the increased time needed to generate a protocol and consent form, the complex volunteer recruitment and selection process adds considerable expense and time to these studies. Limiting volunteers to one per farm or commercial facility extends the number of days of monitoring which adds to the field costs. In the author's opinion it is still unclear as to whether these changes will improve the accuracy of the range of dermal and inhalation measurments in agricultural occupational exposure monitorng studies and the resulting risk assessments.

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Chapter 4

Comparison of Deterministic and Probabilistic Approaches to Modeling Dermal Exposure to Pesticides during Orchard Airblast Application

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Although probabilistic risk assessments have been used for decades in many disciplines, including the regulation of pesticides, mixer/loader and applicator (handlers) risk assessments have been based on deterministic exposure assessments. The current deterministic approach has some major limitations, the most important of which is that the calculated exposures correspond to an unknown percentile of the real exposure distribution (*i.e.*, the distribution of possible exposures over a particular scenario). Exposures may be under-predicted or over-predicted, resulting in risk assessments that do not inform risk managers as to whether the modeled scenario is sufficiently conservative (protective) or unrealistically over-conservative. In a series of numerical examples, the current US Environmental Protection Agency (EPA) approach was compared with more conservative deterministic approaches and with probabilistic approaches for hypothetical, but realistic, orchard airblast mixer/loader and application scenarios. The major determinant of dermal exposure was μg/Lb active ingredient (AI) handled as measured in exposure monitoring studies. This parameter was modeled deterministically with the 50th percentile (approximating the current EPA approach), arithmetic mean, 95th percentile, an upper bound on the 95th percentile and conventional deterministic values for the other model parameters. The probabilistic models were based on lognormal distributions of μg/Lb AI along with distributions for the other parameters.

In Non-Dietary Human Exposure and Risk Assessment; Krolski, M., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2010.

Deterministic estimates of exposure (µg/kg BW/day) corresponded to the 83rd to 99.9th percentiles of the probabilistic distributions. Assuming that a probabilistic assessment best estimates reality, these differences have significant implications for risk assessment as the basis for decisions by risk managers, who would be better informed by probabilistic exposure assessments.

Background

The Agricultural Handlers Exposure Task Force (AHETF) is a consortium of pesticide registrants that is generating a database of dermal and inhalation rates of exposure for pesticide mixer/loaders and applicators. The rates of exposure are commonly known as "unit exposures": µg/Lb AI. The Agricultural Handlers Exposure Database (AHED®) will replace the existing Pesticide Handlers Exposure Database (PHED). Both are based on the consensus that one active ingredient may be used as a surrogate for other active ingredients as long as the formulation type (for mixing/loading) and the equipment used are similar.

In the current regulatory approach, exposure is estimated deterministically:
*Exposure (µg/kg BW/day) = (Lb AI/Acre) * (Acres/day) * (µg/Lb AI handled) / kg bodyweight (BW),* where

Lb AI/Acre—Maximum label rate;
Acres/day—High end productivity estimate;
µg/Lb AI handled—a single 'conservative' value from PHED;
70 kg BW or 60 kg BW—standard man or woman.

The EPA and Canadian Pest Management Regulatory Authority (PMRA) currently use a central tendency value (~50th percentile) from PHED for µg/Lb AI. The California Department of Pesticide Regulation (DPR) uses different high end estimates for acute and longer-term exposures (*1*).

The current deterministic algorithm has some major limitations, the most important of which is that the calculated exposures correspond to an unknown percentile of the real exposure distribution. Exposures may be under-predicted or over-predicted, resulting in risk assessments that do not inform risk managers as to whether the modeled scenario is sufficiently conservative (protective) or over-conservative. This problem may be mitigated by using probabilistic exposure assessments (PEA). Rather than using a deterministic point estimate for each of the above parameters of exposure, a distribution of values can be substituted for each parameter. By iterating the model and randomly sampling a value from each parameter distribution, an exposure distribution can be generated. A subsequent sensitivity analysis can identify the exposure drivers and, if necessary, may be used to identify areas where exposure mitigation could be most effective.

Probabilistic analyses have been used in many disciplines for decades. EPA issued an excellent guidance document in 1997 (*2*). In October 2009, EPA issued an external draft document that provides a thorough discussion of probabilistic risk analysis (PRA), along with copious citations, internet links, and summaries of eight EPA case studies (*3*). Although EPA has used PRA in some areas of pesticide risk assessment (most notably, dietary exposure and risk assessment), it has not used PRA in agricultural occupational exposure risk assessment.

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The purpose of the following numerical experiments was to compare dermal exposure estimates for mixer/loaders and open-cab airblast sprayer applicators using existing and likely alternative conventional deterministic algorithms and probabilistic algorithms.

Models

A hypothetical, but realistic, orchard airblast application and supporting mixing/loading was parameterized as follows.

Lb AI/Acre

Deterministic maximum label rate of application: 4.0 Lb AI/A. Products are often used at less than the maximum label rate: 1.25 – 4.0 Lb AI in a triangular distribution with a minimum of 1.25, most likely of 2.0, and maximum of 4.0 Lb AI/A. The distribution may be highly refined with product use records.

A/day

Deterministic 40 A/day. This is a high end estimate that is seldom achieved. Based on conversations with orchardists, 30 A/day is considered a good day. Also, different products may be used in different blocks on the same day, resulting in much lower “acreage exposure.” This was parameterized with a triangular distribution of 5, 30 and 45 A/day. The distribution may be highly refined with product use records.

µg/Lb AI

The conventional estimates are based on PHED. Unfortunately, PHED estimates are not well suited to probabilistic analyses. The exposure estimates are a hodgepodge of different dosimetry of differing completeness for various combinations of body parts/regions, making the characterization of a distribution very uncertain. Alternatively, the observations in AHED are based on the same type of passive dermal dosimetry in each study, with complete records for each body region. The dosimetry was whole body long underwear worn under long pants and a long-sleeved shirt; detergent-water handwashes of hands under chemical-resistant gloves; and wiping of the face and neck with detergent-water-moistened gauze pads and no head protection. The clothing ensemble of interest was socks, shoes, long pants, long sleeves, chemical-resistant gloves, and no protective head covering. The AHED unit exposures were used in the fashion likely to be used by North American regulatory agencies in deterministic algorithms for acute exposures (1 day):

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50th percentile;

Arithmetic mean;

95th percentile; and,

DPR: the 95% upper confidence limit on the 95th percentile. In this case, an estimated multiplier of 3.7X the arithmetic mean.

The AHED mixer/loader scenario field work for common dry flowable formulations was complete (*4*), with records for 25 person-days of monitoring (monitoring units, individual handlers [MU]). The µg/Lb AI was well characterized by a lognormal distribution. A log-probability plot was linear with a regression r^2 of 0.96. For this stochastic parameter, one could use a distribution based on the observed mean and standard deviation or a fitted distribution. In this case the former distribution was used. It was truncated at 1 and 222 µg/Lb AI, near the lowest observed value and at the mean plus six standard deviations.

The AHED open-cab airblast scenario was incomplete, but does contain records for 23 MUs. Additional data generation is planned. The interim data set was considered to be sufficient for these numerical experiments, as completion of the planned 30 MUs is unlikely to alter any conclusions that may be reached here. The µg/Lb AI was well characterized by a lognormal distribution. A log-probability plot was linear with a regression r^2 of 0.96. As for mixer/loaders, the distribution was characterized by the arithmetic mean and standard deviation. It was truncated at 50 and 17950 µg/Lb AI, near the lowest observed value and at the arithmetic mean plus six standard deviations.

Kg BW

Deterministic 70 kg male. Dermal exposure was not correlated to bodyweights of the 48 mixer/loaders and applicators, which were well characterized as a normal distribution. Because this distribution can return negative and unrealistically high kg BW, the normal distribution was truncated at 50 kg and 135 kg (the lowest and highest observed values).

Repeated values of the stochastic parameters were sampled independently. The different parameters are not known to be correlated. All computations were done in Microsoft Excel 2003 with @RISK Professional 4.5, an add-in developed by Palisade Corporation. Note that @RISK accepts arithmetic means and standard deviations to generate the lognormal distributions noted above. Although the models stabilized at ~2000 iterations in Latin Hypercube Sampling, 50000 iterations were run to obtain better estimates of upper percentile exposures.

Results

The purpose of these numerical experiments was not to generate dermal exposure values to be used in formal risk assessments. The purpose was to compare the results from several approaches to obtaining such numbers. Assuming that the distributions in the probabilistic scenarios were known with confidence, and that the scenario was well characterized by the probabilistic

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exposure assessment, the exposures calculated from the deterministic algorithms were also reported as their corresponding percentiles of the PEA. The results Tables show how use of the 50th percentile, arithmetic mean, 95th percentile µg/Lb AI, or the DPR upper bound, differs from a more realistic PEA.

As shown in Table I for mixing/loading, the mean dermal exposure in the PEA corresponded to approximately the 70th percentile of PEA. Since the predicted exposures were lognormally distributed, this was not an unusual finding. Dermal exposures deterministically calculated from µg/Lb AI at the 50th percentile, mean, 95th percentile, and an upper bound corresponded to approximately the 90th, 95th, 99.4th and 99.9th percentiles of the PEA, respectively. As shown in the sensitivity analyses by multiple regression and rank correlation (Table II), the exposure driver was clearly µg/Lb AI handled. The other parameters were of smaller magnitude, but large enough to be meaningful.

Table I. Comparison of mixing/loading models

Parameter	*50th*[1]	*Mean*[1]	*95th*[2]	*UB*[3]	*Probabilistic parameters*	
Lb AI/A	4	4	4	4	2.42	Triangular (1.25, 2, 4)[4]
A/day	40	40	40	40	27	Triangular (5, 30, 45)[4]
µg/Lb AI	23	34	80	126	34	Lognormal (34, 37, 1, 222)[5]
kg BW	70	70	70	70	93	Normal (93, 16, 50, 135)[5]
µg/kg BW/day	53	78	183	288	24	Expected Value
Percentile[6]	90%	95%	99.4%	99.9%	0.2	Minimum
					686	Maximum
					24	Mean
					31	SD
					15	50th Pctl
					30	75th Pctl
					54	90th Pctl
					77	95th Pctl
					149	99th Pctl

[1] 50th percentile, arithmetic mean µg/Lb AI. [2] 95th pctl µg/Lb AI from lognormal distribution. [3] Upper bound µg/Lb AI, per DPR. [4] Minimum, most likely, maximum. [5] Arithmetic mean, SD, minimum, maximum. [6] Corresponding percentile of probabilistic model.

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Table II. Sensitivity analysis for mixing/loading

	Probabilistic	
Parameter	*Regression*	*Correlation*
µg/Lb AI	0.853	0.872
A/day	0.241	0.341
Lb AI/A	0.186	0.232
kg BW	-0.152	-0.186
R-Squared=	0.846	

Table III. Comparison of airblast application models

Parameter	*50th*[1]	*Mean*[1]	*95th*[2]	*UB*[3]	*Probabilistic parameters*	
Lb AI/A	4	4	4	4	2.42	Triangular (1.25, 2, 4)[4]
A/day	40	40	40	40	26.7	Triangular (5, 30, 45)[4]
µg/Lb AI	685	1400	3660	5180	1325	Lognormal (1400, 2560, 50, 17950)[5]
kg BW	70	70	70	70	93	Normal (93, 18, 50, 135)[5]
µg/kg BW/day	1566	3200	8366	11840	919	Expected Value
Percentile[6]	83%	94%	99.1%	99.7%	9	Minimum
					29212	Maximum
					960	Mean
					1556	SD
					444	50th Pctl
					1057	75th Pctl
					2274	90th Pctl
					3563	95th Pctl
					7896	99th Pctl

[1] 50th percentile and arithmetic mean µg/Lb AI. [2] 95th percentile µg/Lb AI. [3] Upper Bound µg/Lb AI, per DPR. [4] Minimum, most likely, max.imum [5] Mean, standard deviation, minimum, maximum. [6] Corresponding percentile of probabilistic model.

Table IV. Sensitivity analysis for airblast application

Parameter	*Regression*	*Correlation*
µg/Lb AI	0.874	0.921
A/day	0.191	0.268
Lb AI/A	0.151	0.185
kg BW	-0.120	-0.1460
R-Squared=	0.840	

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As shown in Table III for airblast application, the mean dermal exposure in the PEA corresponded to approximately the 75th percentile. Since the predicted exposures were lognormally distributed, this was not an unusual finding. Dermal exposures deterministically calculated from µg/Lb AI at the 50th percentile, mean, 95th percentile and an upper bound corresponded to approximately the 83rd, 94th, 99th and 99.7th percentiles of the PEA, res pectively. As shown in the sensitivity analyses by multiple regression and rank correlation (Table IV), the exposure driver was clearly µg/Lb AI handled. The other parameters were of smaller magnitude, but large enough to be meaningful.

All models have variability and uncertainties, generally falling into the categories of parameter variablility, scenario specification uncertainty and model specification uncertainty. The deterministic models do not capture variability, while the input distributions in the PEA do attempt to characterize variability. There is high confidence (little concern for uncertainty) in the scenario characterization and specification of the model parameters. In this case, the mixing/loading and application scenarios are well understood and contain the key model parameters. Other parameters have been exhaustively analyzed by the AHETF and found to be not quantitatively related to exposure: *e.g.*, loads handled, hours exposed, and years of experience, among others. For the exposure parameters derived from observed data (e.g., the "empirical parameters"), uncertainties are of two types. In the deterministic models, parameterizing with mostly high end values yields a result at an unknown location on the exposure distribution. In the PEAs, uncertainties are associated with the usual limitations regarding the size and representativeness of data sets. For an established product, the Lb AI/A and acres/day distributions can be well characterized. As shown in these data, agricultural pesticide handlers tend to be heavier than average. Confidence in the distribution of bodyweights will increase as the database becomes larger. As shown by the sensitivity analyses, bodyweight is of lesser import than most of the other variables, so parameter uncertainty here is not of major concern. The rate of exposure (µg/Lb AI) will always be based on a limited number of observations due to the prohibitive costs of monitoring handlers. However, there is increased confidence in the AHETF data due to experimental design (*5*), rigorous standardization of sampling methodology, quality control practices, quality assurance programs, and adherence to Good Laboratory Practices regulations. Based on experience with previously conducted studies, the exposure estimates and variances in the AHETF studies are not atypical.

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Discussion

The presented probabilistic models were deliberately simple and represent one module of more sophisticated modeling. It is straightforward to incorporate distributions for inhalation exposure (correlated or not correlated to dermal exposure), dermal absorption, mitigation by protective clothing, days/year, years handled, dietary/drinking water exposure, and dose-response, among others. Probabilistic exposure assessments are the foundation for probabilistic risk assessments that can better inform risk managers. The results of these numerical experiments are but a few examples of how PEA and PRA can inform the risk manager in synthesizing toxicity dose-response, percentile exposure, risk, ground-truthing with incident reports, benefits, and the trade-offs of moving to different percentiles of exposure. Note that scenarios must be evaluated and interpreted case-by-case.

It is also important to note that the presented models are useful only for single-day (acute) exposures. Longer-term exposures and potential toxicity are likely to follow Haber's Law or some variant of it: toxicity equals concentration times exposure duration. An agricultural pesticide handler is not likely to be exposed to the same product every day at a high percentile of exposure. The use of deterministic models, and a single-day PEA, as presented here, will greatly over-predict longer-term exposures, resulting in unrealistically low predicted margins of exposure or high cancer risks. As a consequence, products could appear to be "too risky" and could be denied registration, perhaps unnecessarily depriving agriculture and the public of useful pest management tools. While it might be tempting to simply use the arithmetic mean exposure from a PEA in a short-term, intermediate-term or long-term PRA, that too would over-predict doses from typical intermittent exposures.

Again, PEA allows for the straightforward modeling of intermittent exposures of any selected frequency and duration along with daily dose-averaging consistent with the reference animal toxicology studies. Dose-averaging can be a simple rolling average or it may incorporate pharmacokinetic information. In any case, longer-term PEAs can better inform risk managers when considering longer-term exposures and the relevant toxicology.

The same principles used in these numerical experiments can be readily applied to postapplication reentry exposure. The Agricultural Reentry Exposure Task Force has recently completed a database of dermal exposures for many common tasks (*e.g.*, hand-harvesting tree fruits). The parameters in reentry exposure modeling can be data-rich.

In Non-Dietary Human Exposure and Risk Assessment; Krolski, M., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2010.

Acknowledgments

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Chapter 5

Experimental Design in the AHETF Exposure Monitoring Program

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The Agricultural Handler Exposure Task Force (AHETF) is currently conducting agricultural worker pesticide exposure monitoring studies to populate a new exposure database (AHED®). The design of these studies has been a cooperative effort by AHETF and several regulatory agencies. When completed, this database will be used by both industry and regulatory agencies to assess worker exposures for a standard set of handling scenarios. For each agricultural handling scenario of interest, the AHETF monitoring program obtains a set of experimental monitoring units (MUs) that simulate individual worker 'handling days'. The experimental MUs are quite costly, averaging approximately $50,000 each. Therefore, the experimental design of each scenario attempts to obtain a set of MUs that characterizes important regulatory aspects of worker exposure in as cost-effective a manner as possible.

Introduction

In the late 1990s, pesticide regulatory agencies and the pesticide industry agreed that new exposure monitoring data were needed to replace the limited data in the Pesticide Handler Exposure Database (PHED) (*1*). Despite limitations in quality and breadth of data, PHED was an important component of early-tier agricultural worker exposure assessments conducted by multiple regulatory agencies as well as the pesticide industry. In 2001, the Agricultural Handler Exposure Task Force (or AHETF), was formed to develop a new generic

In Non-Dietary Human Exposure and Risk Assessment; Krolski, M., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2010.

exposure database and conduct exposure monitoring studies to populate it. When completed, this new Agricultural Hander Exposure Database (AHED®) would be used by the pesticide industry, the EPA, and other regulatory agencies for assessing occupational exposures experienced by workers who mix, load and/or apply agricultural chemicals. Since 2001, the AHETF and the Joint Regulatory Committee (JRC), comprised of members from EPA, the California Department of Pesticide Regulation (CDPR), the United States Department of Agriculture (USDA), and the Pest Management Regulatory Agency (PMRA) of Canada have been involved in the design and conduct of exposure monitoring studies for AHED. More recently, the EPA's Human Studies Review Board (HSRB) has also provided considerable input to the design of these studies.

Ultimately, the new exposure monitoring data in AHED should be able to characterize daily pesticide exposure of agricultural workers for a group of pre-defined pesticide handing scenarios. Each scenario is a set of related tasks associated with a particular type of agricultural chemical handing. Examples of scenarios would be 'closed-cab airblast application', 'open pour mixing and loading of liquid formulations', and 'backpack application to utility rights-of-way'.

This chapter summarizes those principles and procedures that have been developed since 2001 and that are now being used to design studies for the AHETF exposure monitoring program. First, background concepts important to understanding the scenario and its relevant components are presented. Next, the AHETF's experimental procedures for characterizing exposure within a scenario are outlined. Lastly, the approach used to find a reasonable number and configuration of experimental units is described.

Background Concepts

This section describes the handler-day, the basic unit of an agricultural handler scenario. Handler-days possess many key characteristics of interest, the most important being the worker's actual exposure to a pesticide. The aggregation of all handler-days in the scenario results in the distribution of exposure normalized by some measure of potential pesticide contact. Specific aspects of this generic normalized distribution are of primary interest to both regulators and pesticide registrants.

Handler-Days

A handler-day (or HD) is the occurrence of a single worker performing scenario-specific tasks over a single workday. For experimental design purposes, the HD is considered the basic unit of a scenario. That is, the scenario can be thought of as an infinitely large collection (or 'population' in statistical terminology) of all HDs that might ever occur.

Every HD is associated with a large set of characteristics. These might include, but are not limited to, such things as:

- Location and date of the HD
- Crop or other site use characteristics
- Identity of worker and worker characteristics
- Environmental characteristics
- Active ingredient used
- Amount of active ingredient handled
- Formulation used
- Product packaging
- Equipment used
- Worker's exposure to active ingredient

The great diversity of possible HD characteristics means that every HD is expected to be unique in some respect.

From a regulatory standpoint, the most important HD characteristic is the worker's dermal exposure to the active ingredient. In particular, interest is often focused on the expected levels of this exposure associated with use of a new, unregistered, active ingredient, or a new use pattern for an already registered active ingredient. Worker exposure is also special because it is 'caused', directly or indirectly, by other HD characteristics.

Genericness and Normalized Exposure

With respect to exposure, some handler-days in the scenario are considered interchangeable. A very important principle for regulatory purposes is that, all else being equal, exposure should be independent of the particular active ingredient used. Exposure is considered more of a physical rather than a chemical process, at least for chemicals that are not highly volatile. Thus, the chemical properties and therefore identity of the active ingredient are irrelevant with respect to exposure.

Another key HD characteristic is the amount of potential active ingredient contact, or PaiC. PaiC is defined as the amount of active ingredient that the worker could potentially contact in a workday during the performance of scenario-specific tasks. For example, an airblast applicator could potentially come into contact with all of the pesticide he applies. Thus, his PaiC is simply the total amount of active ingredient handled (or AaiH) during the workday.

For other scenarios, PaiC could be much smaller than AaiH. For example, a mixer/loader using a closed system might only have the potential to contact a small volume of active ingredient during equipment coupling and uncoupling operations. In theory, if the pesticide were completely isolated from the worker then there would be zero potential for contact. In practice, however, PaiC is unlikely ever to be exactly zero since there is probably some potential for 'background' contact with contaminated surfaces, dust, etc.

For the most part, worker exposure is expected to be a consequence of the PaiC level. For example, one worker using a relatively dilute formulation of a pesticide for a longer period of time might handle the same amount of active ingredient as a second worker using a more concentrated formulation for a shorter period. Assuming similar handling behaviors and other environmental factors, the two workers' exposures are expected to be equivalent, on average. More generally, it is assumed that, all else being equal, a worker's exposure is directly proportional to an appropriate measure of PaiC. Such a relationship might not hold for extremely large levels of contact when skin saturation, for example, could become an issue. But for practical levels of PaiC, proportionality is a reasonable assumption.

When the level of PaiC is known, then HD exposure can be expressed relative to this contact potential. For instance, normalized dermal exposure (NDE) is defined as the ratio:

$$\begin{array}{c}\textbf{Normalized Dermal}\\ \textbf{Exposure (NDE)}\end{array} = \frac{\begin{array}{c}\textbf{Amount of active ingredient}\\ \textbf{deposited dermally}\end{array}}{\textbf{Measure of potential a.i. contact}}$$

When a reasonable measure of PaiC is the amount of active ingredient handed (AaiH) by the worker, then

$$\textbf{NDE} = \frac{\boldsymbol{\mu}\textbf{g of active ingredient deposited dermally}}{\textbf{lbs of active ingredient 'handled'}}$$

Whenever AaiH is used as the normalizing factor, the term 'unit exposure' is often used synonymously with normalized exposure.

Taken together, the above assumptions mean that with respect to normalized exposure, HDs that have different active ingredients or different levels of potential contact with active ingredients are interchangeable, all else being equal. It is in this sense that knowledge about normalized exposure in a scenario can be considered 'generic' knowledge applicable to any active ingredient or any PaiC level.

The Normalized Exposure Distribution

As noted above, each possible scenario HD is expected to be essentially unique with respect to its full set of characteristics. However, many of the HDs might be very similar with respect to a single characteristic such as normalized exposure. If the normalized exposure for every possible HD in the scenario could be determined, the values might appear as the distribution illustrated in Figure 1. The possible normalized exposure values (NDE, for example) are shown along the horizontal axis. The height of the figure represents the proportion of HDs that have any particular NDE value. Many of the possible HDs should have NDE values in a middle range that can be thought of as 'typical'. The frequency of HDs will then tend to drop off for NDE values that are farther and farther away from this typical range. Considerable experience with agricultural handler exposure suggests that the rate of the frequency drop will occur much more slowly for larger NDE values than for smaller values. In most cases, this 'positively-skewed' pattern of NDE can be well approximated mathematically by a log-normal distribution (*2*).

Because normalized exposure is generic with respect to active ingredient and PaiC, its distribution can inform regulators about possible worker exposures resulting from the use of an existing or new chemical. For example, given the application rates proposed for a new chemical, workers might be expected to handle (i.e., be in potential contact with) no more than 30 lbs of active ingredient per day. If every possible value in the NDE distribution were multiplied by 30, a new distribution of dermal exposures is obtained. This derived distribution represents all possible dermal exposures under the scenario for workers handling exactly 30 lbs of the new active ingredient per day. From this distribution, regulators could then judge whether or not these potential 'worst case' worker exposures pose an unacceptable risk for that particular active ingredient and handling scenario.

Of course, this derived distribution is only a construct that is useful for regulatory purposes. It is unlikely to be the exact distribution of exposure for workers handling 30 lbs/day of active ingredient. This is because any particular pesticide might not 'experience' all possible HD conditions that exist over the scenario. For example, suppose that the new chemical considered above will never be applied in the Northwestern US. However, the normalized exposure distribution for the scenario includes HDs that do represent application in the Northwest. Therefore, the predicted exposure distribution for a worker handling 30 lbs/day will be based on some HDs with characteristics that would not occur in practice. In addition to geography, a particular pesticide might also be restricted to specific equipment types, crops, seasons, etc. The fact that the complete scenario distribution has such 'extra' characteristics may or may not impact exposure. If it does, however, then the generic distribution based on the entire scenario will tend to have greater variation than the actual distribution for any particular active ingredient.

Obviously, any scenario's complete normalized exposure distribution is a theoretical concept that would be impossible to determine. As described in the following section, normalized exposure will only be available from a small set of experimentally-obtained HDs. This can only yield partial information about the distribution. Fortunately, knowledge of the complete distribution is unnecessary. From a practical regulatory standpoint, it is only critical to know something about the 'typical' and the 'larger' normalized exposure values (Figure 2). The larger normalized exposure values (multiplied by PaiC) permit regulators to assess risk that might result from short-term (i.e., daily exposure) toxicity. In contrast, the 'typical' exposure levels may be more relevant to assessment of longer-term toxicity driven by sequential exposures. For example, regulators sometimes estimate a worker's longer-term exposure as the scenario average exposure.

Experimental Characterization of Normalized Exposure

The AHETF monitoring program characterizes normalized exposure for a scenario by constructing and monitoring a set of synthetic handler-days. The general objective is to have these synthetic HDs capture a large amount of the diversity in conditions that are believed to affect exposure.

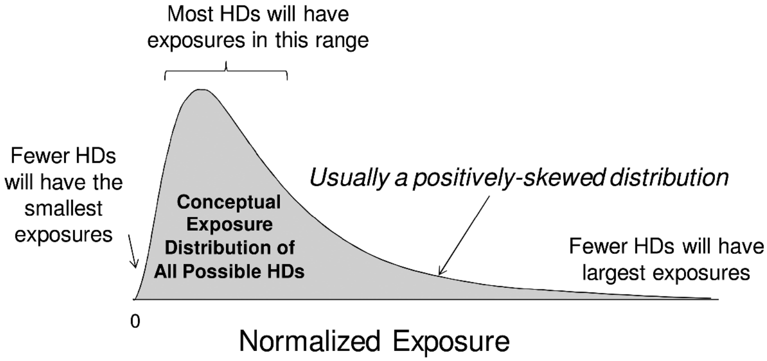
Most HDs will have exposures in this range
Fewer HDs will have the smallest exposures
Conceptual Exposure Distribution of All Possible HDs
Usually a positively-skewed distribution
Fewer HDs will have largest exposures
0
Normalized Exposure

Figure 1. Expected form of the distribution of normalized exposure deriving from all possible HDs in the scenario

Experimental Monitoring Units

A monitoring unit (MU) is the experimental analog of a handler-day. For each scenario, a small set of MUs is obtained and used to characterize the generic normalized exposure distribution. An MU consists of an actual worker who is monitored for exposure to a particular active ingredient while performing scenario-specific tasks representing a complete workday. Each MU should be thought of as a partially synthetic HD that is designed to closely mimic an actual HD. It is synthetic because, although every MU uses actual workers performing actual scenario tasks, there might still be some limited scripting of these tasks by researchers. In addition, MUs also use ‘surrogate’ active ingredients. That is, the intended active ingredient might be replaced with an equivalent chemical that is more conveniently monitored (e.g., chemically analyzed). The use of such surrogate chemicals is feasible because active ingredients are considered interchangeable with respect to exposure.

Experimental monitoring units in the AHETF monitoring program are very expensive. Although exact costs will vary somewhat by scenario, the most recent estimates are that the average cost per MU is approximately $50,000. A substantial portion of this cost is due to activities involved with the recruitment of workers and selection of HD conditions at field locations. Clearly, given such large experimental costs, the number of MUs possible per scenario is limited.

Obtaining a Diverse Set of Monitoring Units

As noted above, a small set of expensive MUs is constructed that mimics the characteristics of a specific group of HDs. How are the HDs (or, more precisely, the HD characteristics) chosen from among the effectively infinite set of HDs that might occur for a particular scenario? Trying to sample randomly from such a conceptual population is impractical and wasteful of the available MUs. However, some practical knowledge should always be available regarding at least the ranges of key characteristics possessed by HDs in the scenario. By using such knowledge, it is feasible to construct MUs that capture the diversity of HD characteristics expected to impact normalized exposure. Such ‘diversity

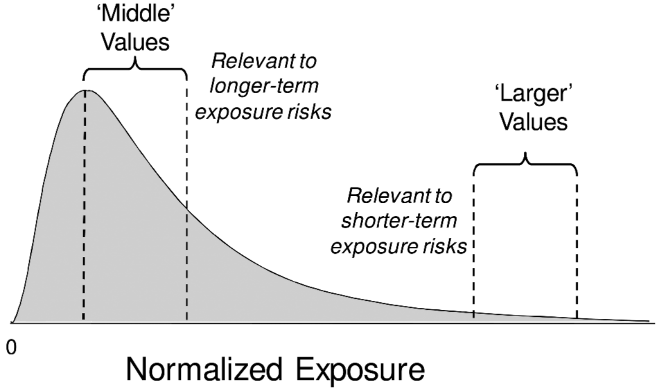

Figure 2. Aspects of the normalized exposure distribution of special regulatory interest

selection' of HD characteristics is the approach being used by the AHETF to construct MUs for scenarios.

When multiple configurations of HDs are possible that might yield equivalent diversity, the MU construction process does incorporate random selection to choose some MU characteristics. The use of such random elements in the selection process whenever feasible serves to reduce the impact of unconscious biases that are possible whenever there is purposive selection of conditions.

Because characteristics that impact normalized exposure are being emphasized in the selection process, the resulting MUs will tend to capture diversity in normalized exposure throughout the scenario. As illustrated in Figure 3, this means that, on average, normalized exposure values for a set of MUs will look more 'spread out' than would the actual scenario distribution of normalized exposure. As a result, the 'larger' values of normalized exposure found in the MUs will tend to overestimate their analogues in the generic scenario distribution. For regulatory purposes, however, over-prediction of exposure is rarely of concern since it is conservatively 'protective' of workers when used in an exposure assessment. In contrast, 'typical' values of normalized exposure for the set of MUs should be closer to the analogous 'typical' values in the generic scenario distribution.

The AHETF Monitoring Program tends to formally diversify the MUs with respect to three key HD characteristics: geography, worker, and PaiC. Each of these characteristics are considered 'meta-factors'. That is, they are really a surrogate for a large array of HD characteristics that are likely to impact normalized exposure, although the direction of this impact cannot be predicted. Thus, selecting a set of actual HDs that differ with respect to these three meta-factors is an efficient starting point from which to construct a diverse set of MUs.

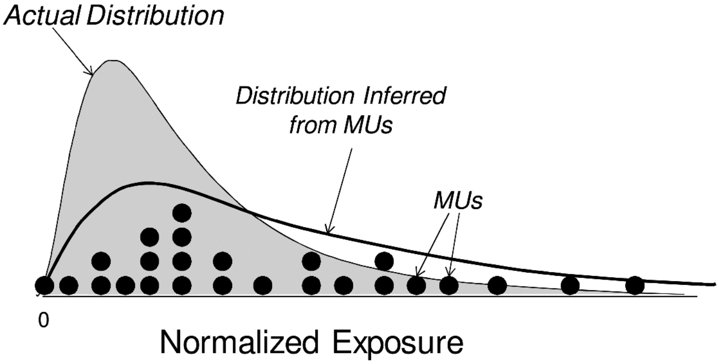

Figure 3. The apparent normalized exposure distribution inferred from MUs obtained by 'diversity selection'

Geographic (and Temporal) Diversity

Any two handler-days that occur in different geographic areas and at different times (e.g. months, years, etc.) are expected to exhibit different levels of exposure. The cause for such differences is unlikely to be the spatial or temporal positions per se. Rather, these two widely-separated HDs are expected to differ in a myriad of ways, many of which have some indirect impact on exposure. For example, agricultural workers from different regions might utilize slightly different equipment and techniques, and seasonal differences can impact the size or foliage conditions for treated crops. Thus, spreading out the MUs geographically and temporally is simply a convenient way to influence many factors at once.

Clearly, maximum diversity would be obtained by conducting every MU in a different geographic location. However, as described below, there is an extremely large overhead incurred for each new monitoring location. Therefore, maximum geographic diversity would require very large costs and thus reduce the number of scenarios that could be characterized by the AHETF Monitoring Program. For this reason, the AHETF only distributes local clusters of MUs geographically. The spatial and temporal extent of a cluster is not rigorously fixed. However, MUs within a cluster will typically range over several counties and be conducted over the course of one to two weeks.

The process of distributing the clusters involves more that simply varying their geographic position. For example, the possible areas of the US might be first partitioned into climatic 'growing' regions. Clusters would then be distributed so that no two are located in the same growing region. In addition, distributing clusters to different geographic regions often means that clusters are obtained at different dates as well. In fact, different clusters of MUs for the same scenario might even be obtained in different seasons or even different years. Thus, a great amount of diversity is expected between clusters. As a result, normalized exposure levels for two MUs in the same cluster are expected to be more similar, on average, than for two MUs in different clusters. In other words, some intra-cluster correlation (or ICC) of normalized exposure is quite likely.

Worker Diversity

Different workers are expected to exhibit substantial differences in normalized exposure, even when performing the same pesticide handling tasks. Consequently, every MU will utilize a different worker. There also may be some similarities in handling behaviors among workers of the same employer. To reduce the possibility of such correlations, no two workers in a cluster are selected from the same local organization (e.g., same grower or same commercial application company).

For each cluster, a comprehensive and costly recruitment process is used to locate participating workers. Briefly, an attempt is first made to list every farm or application company in the targeted geographic area (e.g., a group of adjacent counties) that might meet certain HD requirements. This list is then screened in random order until an adequately-sized working pool of farms/companies that actually meet the requirements is obtained. Detailed interviewing is then conducted to further characterize the potential workers and expected HDs within the randomly selected working pool and find workers that are willing to participate in an exposure study. Finally, a cost-effective configuration of farms/companies, workers, and HDs is selected from which to construct MUs. Whenever there are multiple equivalent workers or HDs available for an MU, one of these is selected at random.

This complex worker recruitment process results in an extremely large overhead cost per cluster. Its primary advantage is that it ensures that the set of workers and HDs being considered is a reasonable cross section of the local worker population. In addition, the incorporation of random sampling elements in the selection process whenever feasible reduces the likelihood of MU selection biases.

Diversity in Potential AI Contact

The third characteristic employed in the formal diversity selection is the level of potential active ingredient contact (PaiC). First, the measure of PaiC considered most appropriate for the scenario is determined. In many cases, this measure will be the amount of active ingredient handled (AaiH) by the worker. Then, the practical range of this PaiC measure is determined. This range is based on knowledge about the scenario and reasonable assumptions concerning typical application rates, acres treated, and work practices. The practical range is generally narrower than the maximum possible PaiC range. For example, extremely small levels of PaiC, although possible, can result in exposure levels too small to be practically measured. At the other extreme, an HD using an extremely large level of PaiC might be difficult to find in the working pool. Once the practical range has been defined, the PaiC values targeted for each MU within each cluster are simply spread out over this range. A common design is to define logarithmically spaced partitions (or ‘strata’) of the PaiC range and to obtain one MU per partition within each cluster.

It is reasonable to ask why diversity in PaiC is desirable if interest is centered on normalized exposure. As noted above, normalized exposure should be independent of PaiC. This is likely true for PaiC, per se, but not for other characteristics that might be associated with PaiC in practice. For example, it is likely that HDs using different levels of PaiC will also employ different equipment or result in different worker behaviors. It is these other characteristics, not the level of PaiC itself, that are likely to impact normalized exposure. Therefore, diversifying PaiC also diversifies the MUs with respect to all these characteristics simultaneously.

As described below, one (secondary) quality objective of the collected data is that it should permit a limited examination of the assumption that exposure is proportional to the measure of PaiC used. The statistical power of this comparison is improved when the set of MUs within each cluster spans the practical range of PaiC values.

Number of Clusters and Monitoring Units

The process the AHETF uses to select HD conditions and construct MUs has been described above. But nothing has been said about how many MUs are needed for a scenario and how those MUs should be partitioned into clusters. In the purest sense, statistical methods cannot directly establish sample size for this diversity selection process. Statistical theory can only predict how larger sample sizes improve ‘quality’ when the process is, or is assumed to be, random. For example, suppose it is desired to describe the mean weight among a large group of people by using a random sample of N persons. In this case, statistical theory can be used to determine how close, on average, the sample mean is to the true mean weight for any possible value of N. The value of N that gives an acceptable accuracy could then be chosen. However, if the N persons are purposively chosen so they represent a diverse mix of height, gender, race, and body style, then statistical theory does not apply.

Nevertheless, a reasonable method of determining a sample size is still possible. The approach used by the AHETF is first to define a random sampling situation that is analogous, in some important respects, to the way the HDs are used to construct MUs. Then, because this theoretical analogy involves random sampling, statistical theory can be employed to determine sample sizes.

Figure 4 illustrates this correspondence between the ‘real world’ selection of MUs and the random sampling approach used as a theoretical analogy. As shown in Figure 4A, the AHETF actually selects a set of HD characteristics from among all of those considered possible under the scenario. These characteristics are used to construct MUs which, when monitored, provide normalized exposure measurements. These data are then used to characterize the diversity of normalized exposures in the scenario.

The corresponding theoretical analogy is summarized in Figure 4B. Here we assume that the scenario consists of a large population of HDs. From this population, a random sample of HDs is selected and monitored for exposure. (These monitored HDs are then become MUs.) The assumed sampling process might be more complicated than simple random sampling. The resulting sample of normalized exposures is then used to characterize the distribution.

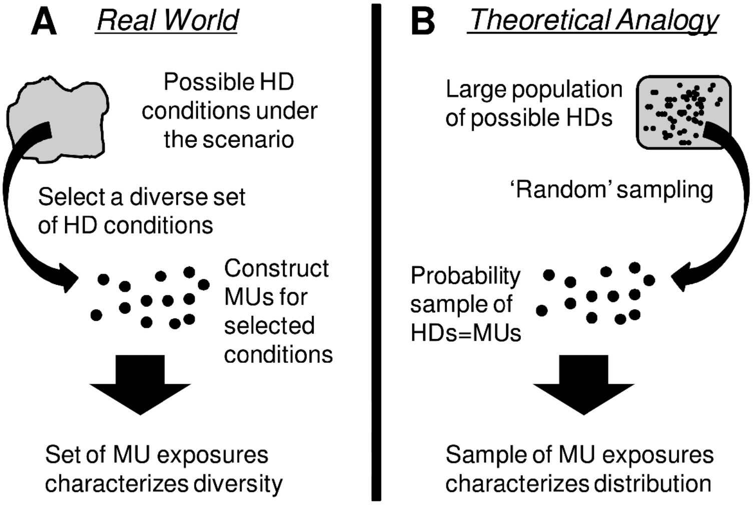
A Real World
Possible HD conditions under the scenario
Select a diverse set of HD conditions
Construct MUs for selected conditions
Set of MU exposures characterizes diversity
B Theoretical Analogy
Large population of possible HDs
'Random' sampling
Probability sample of HDs=MUs
Sample of MU exposures characterizes distribution

Figure 4. Correspondence between the actual 'real world' for obtaining MUs and the theoretical analogy used to determine sample sizes

The process used by AHETF to determine reasonable sample sizes has three basic components:

1. Specify a sampling 'reference' model that is a random sampling analog to the way MUs are actually obtained.
2. Define objective(s) that the resulting data should meet to be of value if the reference model was actually used.
3. Determine what sample sizes would be necessary to meet the stated objectives if the reference model were used.

The sample sizes that would achieve the desired objectives if the random sampling reference model were appropriate are then used in the actual monitoring program. It is felt that this approach provides objective guidance to research personnel and is a reasonable alternative to choosing either an arbitrary number of MUs or choosing a number based on cost considerations only.

Random Sampling Reference Model

The reference model used for most AHETF scenarios is illustrated in Figure 5. In this model the distribution of normalized exposure values over all possible handler-days in the scenario is assumed to follow a lognormal distribution with geometric standard deviation equal to GSD. The reference model further assumes that HDs are randomly sampled from this scenario population in two distinct stages. The first stage consists of randomly sampling N_C clusters of HDs out of all possible clusters. This stage is the analogue for the selection of a diversity of geographic locations for clusters. The second stage of sampling in the reference model assumes that N_M HDs are randomly selected from within each of the first stage clusters. This second stage is the analogue for all the diversity and random selection that actually occurs at a particular geographic location. Two stages of random sampling permit the reference model to include the likely correlation among normalized exposures for HDs in the same cluster (i.e., the ICC). These 'randomly sampled' HDs, when monitored, are then considered MUs.

It must be emphasized that the random sampling reference model shown in Figure 5 does not describe how the MUs are actually obtained. It is only a convenient analogy for the real selection process and is only used to provide guidance about sample sizes. Certainly, random sampling structures that are much more complicated than shown in Figure 5 could also be assumed. However, this would require that additional parameters besides GSD and ICC be estimated or assumed. Since random sampling of HDs is only an analogy for the actual MU construction process, additional complexity seems unwarranted.

Benchmark Objectives

Using the random sampling reference model it is now possible to consider a limited set of objectives that can be attained by varying the sample size. These are considered merely 'benchmark' objectives because (1) they apply to the reference model only and (2) do not cover everything that users of the monitoring data might want to know. Currently, all AHETF scenarios use the following **primary benchmark objective**:

> *If the random sampling reference model were true, the sample size should be adequate so that the geometric mean, arithmetic mean, and the 95th percentile of normalized dermal exposure are accurate to within 3-fold, 95% of the time.*

The geometric and arithmetic means are standard statistics describing the 'middle' or 'typical' values of a distribution. Similarly, the 95th percentile is a commonly-used statistic for 'larger' values. The AHETF, and regulatory agencies advising AHETF, consider a 3-fold accuracy requirement to be a reasonable benchmark for regulatory purposes.

An additional benchmark objective is also considered for those scenarios in which the practical range of PaiC is expected to be large (e.g., over an order of magnitude). This **secondary benchmark objective** can be stated as:

> *If the random sampling reference model were true, the data should provide at least an 80% power for distinguishing a proportional relationship between dermal exposure and PaiC from complete independence of these two quantities.*

The power in this case refers to a test based on the linear regression of log exposure on log PaiC. Here, proportionality would imply a slope of one and independence would imply a slope of zero.

In these two benchmark objectives, dermal exposure refers to 'total dermal exposure', the sum of all separately measured dermal exposure components.

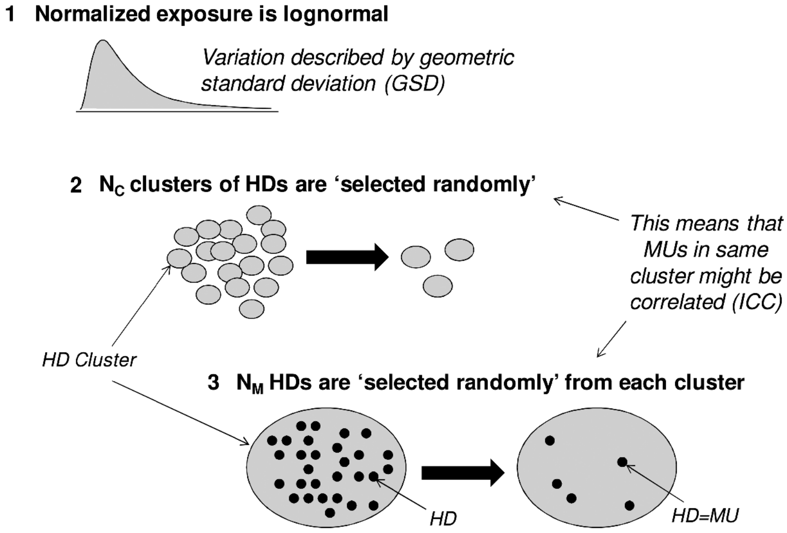
1 Normalized exposure is lognormal
Variation described by geometric standard deviation (GSD)
2 N_C clusters of HDs are 'selected randomly'
This means that MUs in same cluster might be correlated (ICC)
HD Cluster
3 N_M HDs are 'selected randomly' from each cluster
HD
HD=MU

Figure 5. The random sampling reference model used as a theoretical analogy when determining sample sizes

Total dermal exposure is used as the benchmark quantity because it is typically considered the most important single agricultural worker exposure characteristic. However, users of the AHED® database will always have access to values of the individual dermal exposure components, as well as inhalation exposure, for each MU.

Finding Acceptable Sample Size Configurations

For a particular scenario, the number of clusters (N_C) and the number of MUs per cluster (N_M) that satisfy the primary objective are found using Monte Carlo simulation and bootstrapping methods (*3*). Default values of the geometric standard deviation (GSD) and the intra-cluster correlation (ICC) were obtained by examining a limited set of existing monitoring data for various scenarios. These analyses suggested that GSD=4 and ICC=0.3 were reasonable values to use when more specific information was lacking. For the lognormal distribution, knowledge of GSD is sufficient to define the geometric mean, arithmetic mean, and 95th percentile (up to a multiple of the geometric mean).

Trial values of N_C and N_M are first chosen. Then normalized exposure values are simulated for N_C clusters with N_M MUs per cluster. These simulated data are then analyzed to obtain estimates of the geometric mean, the arithmetic mean, and the 95th percentile all relative to their true values. This simulation is repeated 10,000 times and the 95% limits for these relative accuracies calculated. If any statistic has a 95% bound on relative accuracy that is worse than 3-fold, new values for N_C and N_M are tried. This entire process is repeated until a set of N_C and N_M is found that results in 3-fold or better accuracy for every statistic of interest.

Whenever the secondary 'power' benchmark objective is used, the simulated exposure data corresponding to proportionality is also generated for a specified configuration of PaiC. These data are analyzed with mixed-model regression (*4*) and the confidence interval for the slope is determined. The average width of this confidence interval is directly related to the power to distinguish proportionality from independence. When the PaiC levels within each cluster are spread out over the practical range, the power objective is always satisfied whenever the primary ('3-fold accuracy') objective is satisfied. Thus, unless there are unusual design considerations, only the primary objective need be evaluated when determining sample size.

Finding the Best Configuration of N_C and N_M

The simulation approach described above will find a configuration of number of clusters and number of MUs per cluster that satisfy the benchmark objectives. However, there will usually be multiple configurations that do this. For example, when the default variation structure (i.e. GSD=4, ICC=0.3) is used with the random sampling reference model, all the configurations listed in Table I meet the benchmark objectives. In practice, the configuration that is actually used will be the one that is most cost effective or most logistically feasible.

For example, suppose that the estimated cost for obtaining a single-MU cluster is \$150,000 and the cost of a larger 5-MU cluster is estimated to be \$250,000. This implies that the average cost per additional MU is (250,000-150,000)/4 = \$25,000 and that the 'overhead cost' per each additional cluster is 150,000 – 25,000 = \$125,000. In other words, the cluster overhead is five times larger than the expected cost per additional MU. Such a large overhead puts a severe cost penalty on configurations with a large number of small clusters. This is true even though (when ICC>0) more clusters always means fewer total MUs. As shown in Table II, it is this overhead/MU cost ratio that determines which of the sample size configurations in Table I is most cost effective. In the example above, the relative cost ratio of 5 implies that 5 clusters with 5 MUs per cluster is optimal. In contrast, if the cluster overhead ratio were only 1, say, then it would not be as expensive to obtain additional clusters relative to additional MUs. In this case it would be most cost effective to obtain 9 clusters with only 2 MUs each.

Sometimes, the choice of a configuration is based on both feasibility and cost. For example, for some scenarios, there may be too few qualified and willing workers available to construct the number of MUs needed for the most cost-effective configuration. In such cases, one might move up in Table II to a configuration with fewer MUs per cluster. This would mean more clusters and a greater total cost. But it would still be the least costly of the possible configurations.

Table I. Combinations of N_C and N_M that meet the benchmark objectives when GSD=4 and ICC=0.3

Number of Clusters, N_C	*Number of MUs per Cluster, N_M*	*Total Number of MUs*
15	1	15
9	2	18
7	3	21
5	5	25
4	8	32

Table II. Relative cluster overhead costs that determine cost-effective combinations of N_C and N_M

	Most Cost-Effective Configuration	
Relative Cluster Overhead[a]	*Number of Clusters, N_C*	*Number of MUs per Cluster, N_M*
Below 0.5	15	1
0.5 to 1.5	9	2
1.5 to 2	7	3
2 to 7	5	5
Over 7	4	8

[a] Ratio of cluster overhead cost to the cost per additional MU in a cluster.

Summary

The AHETF exposure monitoring program has a clearly defined conceptual and experimental basis. Experimental MUs are obtained through a diversity selection of HD conditions. A random sampling reference model is used as a convenient conceptual analogy of the actual diversity selection process. By coupling this reference model with benchmark objectives relevant to regulatory purposes, reasonable choices for the number and arrangement of MUs can be made. The final sample size determination balances the regulatory objectives with cost-effectiveness.

Acknowledgments

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Chapter 6

Effect of Changes in Human Exposure Regulations on Quality Assurance

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With the new regulations set forth by the US EPA in 40 CFR Part 26, the QAU has needed to adapt to a new set of regulatory criteria in planning, and auditing worker exposure studies using human subjects. This manuascript is a summation of the work that the Agricultural Handler Exposure Task Force QAU has had to deal with while incorporating these new rules. It also addresses the additional amount of work that the QAU must face in auditing these studies.

The role of the Quality Assurance Unit (QAU) has been continually redefined since its official inception in 1978 for pharmaceutical research (*1*) and 1989 for agrichemical research (*2*). Even though the basic responsibilities are defined in the Good Laboratory Practices (GLPs), QA Professionals who perform these responsibilities run into new situations that require rethinking and new approaches to completing the work. Quality assurance is not quality control, nor is it exclusive to GLP-driven studies, but it is a comprehensive approach to the overall research process. In order to provide the best possible oversight for the clients, including the Federal Agencies who ultimately will use the study results for regulatory decisions, the QAU must adapt to new situations and incorporate new paradigms to assure that the quality of any particular study is at its highest level.

The ability to participate in an intensive research program, such as an industrial task force, for example the Agricultural Handler Exposure Task Force (AHETF), can provide a great deal of experience in dealing with the evolution of worker exposure study QA aspects. This includes the incorporation of the new rules outlined in 40 CFR Part 26 (*3*) and Environmental Protection Agency (EPA)

In Non-Dietary Human Exposure and Risk Assessment; Krolski, M., et al.;
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requirements for these studies. The AHETF is a group of pesticide manufacturers and registrants that was formed in December 2001 to develop additional data to better represent actual exposure levels for a wider range of agricultural pesticide handler activities. The mission of the AHETF is to share resources in the design, evaluation, and development of a proprietary agricultural handler exposure database for use in regulatory risk assessment.

In this chapter, the role of the QAU for Worker Exposure research (WEx) will be examined, considering the EPA's new rule for Protection of Human Subjects, as defined in 40 CFR, Part 26. This new rule became effective on February 6, 2006, and covers intentional exposure of persons involved in agricultural research to pesticides. Specifically, this rule prohibits the intentional dosing, or exposure, of pregnant or nursing women and children to pesticides. The specifics of this new rule will not be discussed in this chapter; however, the effects of this new rule in performing quality assurance duties will be.

Just like the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA 40 CFR Part 160) GLPs, this new rule requires compliance with certain aspects of the WEx research. If there is a Federal requirement for research, then it falls upon the QAU to assure that compliance with all regulatory criteria is met. The requirements outlined in Part 26 provide a challenge to the QAU to incorporate the compliance aspect into the normal QA activities on an agrichemical WEx study.

The 40 CFR, Part 26: Protection of Human Subjects is structured in subparts. The major sections of this rule are outlined as follows:

- Subpart A: Basic EPA Policy for Protection
- Subpart B: Prohibition of Research
- Subpart C: Observational Research - Women
- Subpart D: Observational Research – Children
- Subpart K: Basic Ethical Requirements
- Subpart L: Prohibition of Third-Party Research
- Subpart M: Requirements for Submission
- Subpart O: Actions for Noncompliance

From these sections, it is easy to see that this rule addresses the protection of certain subjects and also addresses the ethical aspects of conducting the research. The restrictions addressing the conduct of a study are clearly discussed in sections B, C and D – where pregnant or nursing women and children (under 18) are prohibited from participation in research or observational research programs. The ethical requirements for non-nursing/non-pregnant adults who are eligible for participation are challenging aspects for the QAU to develop suitable means to confirm compliance. One example is that the consent process to register a worker's participation must be conducted in a non-coercive manner, which is interpreted to mean a one-on-one interview and disclosure meeting. This occurs between researchers, usually the Study Director, and the potential subject. The QAU may not observe or participate in this process. This is a challenge to the QAU, who normally observe all study procedures and compare them to documents such as the Study Protocol and written Standard Operating Procedures (SOPs). As the QAU may not evaluate this procedure first-hand, they must find

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a substitute method to assure study management it was actually fulfilled by the research staff.

In addition, many aspects of conducting this research under the new rule occur before the protocol is finalized and the field research is begun. Typically, the QAU doesn't get involved in a study until the final protocol is provided to the QAU (or a draft version is provided) for a GLP compliance review. In order to comply with EPA requirements, the study protocols must be reviewed by the Human Studies Review Board (HSRB). The HSRB, a Federal advisory committee, provides advice, information, and recommendations on issues related to scientific and ethical aspects of human subjects research. The major objectives are to provide advice and recommendations on: a. research proposals and protocols; b. reports of completed research with human subjects; and c. how to strengthen EPA's programs for protection of human subjects of research. The HSRB reports to the EPA Administrator through EPA's Science Advisor.

Additionally, the study protocol, worker consent forms, any information that will be provided to the workers, and even Standard Operating Procedures (SOPs) require a review by an Institutional Review Board (IRB) prior to the conduct of the research. This process will typically begin six months to a year before the field research will actually be conducted.

A general timeline for preparing the protocol and other documentation is typically as follows:

- Registrant develops study plan
- Protocol and information documents are prepared
- All documents undergo IRB review
- All documents submitted to EPA/HSRB
- With approval, study may begin
- Otherwise, revisions will be necessary and whole process starts over.

In other field research, the QAU typically becomes involved with the protocol process after the study plan has been developed and the protocol is submitted to the QAU for a GLP compliance review. With the WEx Protocol timeline, the QAU will need to be involved much sooner in order to assure that IRB and HSRB reviews have been completed *prior* to the finalization of the protocol and supporting documentation. In order to assure these requirements are being met, the QAU will need to communicate with the Study Director and study management during the development process, rather than waiting until the finalization stages.

As a further example of the QAU involvement in monitoring the development of a WEx study, the AHETF QAU has focused on Subpart K "Basic Ethical Requirements." The following list is a summary of the section issues that are verified by the QAU:

- Have all materials submitted to an IRB? [§26.1115(a)(1)]
- Are minutes of IRB meeting(s) documented? [§26.1115(a)(2)]
- Are copies of IRB correspondence maintained? [§26.1115(a)(4)]
- Have SOPs submitted to IRB? [§26.1115(a)(6)]
- Are the potential risks discussed? [§26.1125(a)(1)]

- Have measures been taken to reduce risk? [§26.1125(a)(2)]
- Have the nature of benefits been discussed? [§26.1125(a)(3)]
- Were alternative means of obtaining data explored? [§26.1125(a)(4)]
- Discussed balance of Risk vs. Benefits? [§26.1125(a)(5)]
- All info/documents reviewed by an IRB? [§26.1125(b)]
- Are recruitment procedures documented? [§26.1125(c)]
- Is there a description of Methods of Presenting Information (consent process)? [§26.1125(d)]
- Is there documentation of correspondence? [§26.1125(e)]
- Has official notification from an IRB has been received? [§26.1125(f)]

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It should be evident that the QAU has an expanded role in monitoring the developmental stages of WEx study, rather than just monitoring the conduct of the study itself. The additional time for review, comment and correction to the study protocols, accounting for the details to address the Part 26 requirements, can nearly double the resources that a non-worker exposure studies. These requirements, adding the necessary Part 26 verbiage to a worker exposure protocol and responses to HSRB comments can nearly double the size of the protocol. To illustrate this, the WEx protocols that were written for AHETF studies prior to 2006 were about 25 pages. Adding the HSRB and EPA language increased the protocols to over 40 pages. This is an additional burden on the QAU as the protocols are more complicated and require more time to thoroughly review.

When the field phase begins, the QAU will be concerned with Subparts B "Prohibition of Research", K "Basic Ethical Requirements", and M "Requirements for Submission." The following examples are the specific sections the AHETF QAU monitors:

- §26.203: "Prohibition of Research Conducted…"
    - Assure that female participants are not pregnant or nursing
    - Assure that no participants are under 18 years old
- §26.1117: "Documentation of Informed Consent"
    - Assure no coercion though available documentation only (QAU cannot be present during consent)
- §26.1303: "Submission of Information…"
    - Assure that all appropriate ethical documentation is complete

The QAU involvement with WEx research is not limited to only the development of the protocol and conduct of the study. In many instances, the QAU is responsible for the development and maintenance of the standard operating procedures. After extensive reviews of the AHETF program and documentation, suggestions were presented to the AHETF from the HSRB and EPA over several meetings, that specific ethical requirements be described in written SOPs. The following list is examples of the new procedures drafted and approved by the AHETF:

- Personnel Responsibilities – Ethics Training
- Procedure for Recruiting Study Participants
- Archiving Confidential Worker Information
- Ethical Requirements for Studies
- Recruiting Volunteers
- Worker Health Status
- Pregnancy Testing
- Pesticide Safety Precautions
- Adverse Events Reporting for IRB
- Identification and Control of Heat Stress
- Emergency Procedures for Human Subjects
- Language Considerations
- Informed Consent of Study Volunteers
- Compiling Lists of Potential Growers
- Compiling Lists of Potential Applicators
- Recruiting Eligible Growers/Applicators

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Prior to the establishment of the new rule, there was no need to have such an extensive list of these procedures, as specific SOPs on ethical conduct were not a GLP requirement. Since the oversight provided by the HSRB, both the EPA and industry found it necessary to develop specific written procedures to adequately address common issues, as presented in the aforementioned list of SOPs. These SOPs were developed over several months, in response to comments from the EPA and HSRB. The majority of these SOPs was also reviewed and revised a number of times -- even before being implemented in an AHETF WEx study. The AHETF QAU had to devote a considerable amount of time to develop, review, revise and implement these additional SOPs. For the AHETF program, the QAU spent hundreds of hours over 18 months on just these sixteen procedures.

The main charge to a QAU is to assure study management that all applicable regulatory requirements are met, as well as, assuring that the protocol and specific SOPs are followed. With this new rule in place all worker exposure studies conducted for submission to the EPA must now address specific information in a number of areas, including the protocol and SOPs. With these regulatory requirements and documents in place, the QAU has an obligation to audit and assure compliance for study management, the Registrant, and the EPA. What this means to the QAU is more paperwork. Protocols and final reports have greatly increased in size, which in turn requires more effort to audit. Several SOPs should be prepared to address ethical issues for WEx studies. While there is no requirement that the QAU prepare these SOPs, it often falls to the individual QAUs to manage these documents, which again adds time to review and manage these documents. Finally, the QAU must be flexible in its approach to auditing the new worker exposure study in order to monitor the GLP compliance as well as the ethical treatment of the workers.

One last issue regarding conduct of these studies is the requirement for all research personnel to take an appropriate ethics class (*4*), such as the National Institutes of Health (Protecting Human Research Participants (PHRP)) and/or the Basic Collaborative IRB Training Initiative Course (CITI; The Protection of Human Research Subjects). These are links to both of these on-line training courses at www.nih.gov and www.citiprogram.org. While the QAU generally does not have this direct contact with the worker as the researchers do, such as collecting samples from the worker, the AHETF policy is for all study personnel to have completed such an ethics class, including the QAU personnel. These online classes generally take a few hours to complete and provide a downloadable certificate upon completion.

With the recent implementation of 40 CFR Part 26, there is still a lot to learn and develop between the Registrant, the contract research organization, the QAU, the HSRB and the EPA. Much of the time between 2006 and 2008, for the AHETF, was spent developing procedures, surveying the agricultural community, writing and revising protocols, SOPs, and worker consent and information documentation. No studies were conducted during this period, as HSRB approval was not obtained until all of the study related documentation was clear and satisfactorily addressed all of the details the EPA and HSRB required. This was particularly difficult in that the QAU was writing field SOPs – then revising them – without ever having implemented them in the field.

In summary, adaptation to new study requirements is nothing new in this industry. All research organizations had to adapt to following the GLP regulations when they were implemented in 1989, and now after more than 20 years, many researchers and QAUs may find it hard to imagine conducting these studies without GLPs. It is no different with the Protection of Human Subject regulations now. While there is some difficulty in understanding the new requirements and implementing them into the study design, it is not impossible to do. But there is a cost to be paid. Over time, these requirements will become just as ingrained as the GLPs and we will have to look for the next regulatory change, and adjust to it.

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Chapter 7

Design of an Observational Worker Exposure Study in Commercial Seed Treatment Facilities

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To support seed treatment products manufactured by Bayer CropScience (BCS), an observational study consistent with the Protections for Subjects in Human Research Rule was performed to determine the potential exposure for workers involved in various work functions associated with operating commercial seed treatment systems. Workers at two canola treating facilities in Canada and three corn treating facilities in the United States were monitored during treating, bagging/sewing/stacking, and cleanout activities to determine the nature and amount of exposure to seed treatment chemicals during their normal work activities. This paper describes the general experimental design and the processes used to identify and select the seed treatment facilities and volunteers used in the study.

Introduction

The purpose of this study was to generate dermal and inhalation exposure data for workers who perform commercial oil or grain seed treatment activities with imidacloprid, clothianidin, metalaxyl, and carbathiin (carboxin). One work function was activities involved with the treatment of the seed; such activities include loading and treating. A second work function was processing the treated seed; such activities include bagging/sewing/stacking, and forklift operations. The third work function was the thorough cleanout of the treatment equipment.

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ACS Symposium Series; American Chemical Society: Washington, DC, 2010.

A secondary objective of this study was to select facilities treating canola and facilities treating corn to determine if there is a difference in the exposure potential based on seed type. A third objective was to monitor exposure potential during the treatment of seed with two concurrent active ingredients to investigate the relationship between the amount of active ingredient handled and the exposure potential. A fourth objective was to generate data allowing a comparison of intra- and inter-worker variability in activity related exposure. Data from this study can also be employed generically to support other liquid products for commercial seed treatments which require comparable equipment for application.

The study was designed to fulfill the requirements of the US EPA Series 875: Occupational and Residential Exposure Test Guidelines, and meet all applicable requirements of US EPA 40 CFR Part 26 Protection of Human Subjects and 40 CFR Part 160 FIFRA Good Laboratory Practice Standards. Although not a requirement of the rule, the study protocol was submitted to the EPA prior to experimental start to seek their determination as to whether the study design was observational and not a study involving intentional exposure to a human subject as defined in 40 CFR §26.202(a). The rule requires that protocols for studies involving intentional exposure to human subjects be submitted to EPA prior to study initiation.

Since the study was intended to be observational, and performed in commercial facilities during normal production, the chemicals to be monitored were limited to those registered and in common use at the time of the study. To allow for maximum flexibility in location of the test sites, the study was planned to allow for the use of multiple test chemicals. As most commercial seed treatment incorporated both insecticide and fungicide components, two insecticides and two fungicides registered for use on both canola and corn were included in the study design. This paper describes the general experimental design and the processes used to identify and select the seed treatment facilities and volunteers used in the study.

The active ingredients targeted in this study are present in a number of registered seed treatment products. GAUCHO® 480 and GAUCHO® 600 are formulated products containing the active ingredient imidacloprid, an insecticide labeled for use on canola, rapeseed and corn. PONCHO® 600 FS, a formulated product containing the active ingredient clothianidin, is an insecticide labeled for use on canola, rapeseed and corn in commercial seed treatment facilities with closed transfer systems. PROSPER™ FL, a formulated product containing the insecticide clothianidin and the fungicides carbathiin (carboxin), thiram and metalaxyl, is labeled for use on canola and rapeseed in commercial seed treatment facilities with closed transfer systems. ALLEGIANCE® FL, a formulated product containing the active ingredient metalaxyl, is a fungicide labeled for use on a variety of crops, including corn in commercial seed treatment facilities.

Two additional registered seed treatment products in use at the facilities monitored were found to be relevant because the analytical methodology does not differentiate between mefenoxam, an active ingredient in these products, and metalaxyl, an active ingredient in PROSPER™ FL and ALLEGIANCE® FL. These additional seed treatment products include: (i) Maxim® XL, a formulated product fungicide containing the active ingredients fludioxonil and mefenoxam,

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registered for use on a variety of crops, including corn in commercial seed treatment facilities, and (ii) APRON XL® and APRON XL® LS, formulated product fungicides containing the active ingredient mefenoxam, registered for use on a variety of crops, including corn in commercial seed treatment facilities.

The structures, names, and CAS registry numbers of the active ingredients used in this study are given in Table I.

Study Design

The study was conducted by following a protocol approved by the ethics committee of Bayer CropScience (BCS), the Independent Investigational Review Board (IIRB), PMRA, and USEPA's Office of Pesticide Programs. All amendments to the protocol relevant to the field phase of the study were signed and dated by the Study Director and approved by the IIRB. Deviations from the protocol were documented and communicated to the Study Director and the IIRB and recorded in the raw data.

Study Time Line and Responsibilities

The sequence of acivities involved in study design, planning, recruitment, and field work are outlined below:

1) Review of protocol and consent form/process by the BCS ethics committee.
2) Review of protocol and consent form/process by the IIRB.
3) Observational Study determination by USEPA and PMRA based on protocol review.
4) Identification, initial qualification, and randomization of sites by the Study Director and PFI.
5) Meeting with site management to explain the study goals and methods, and gain approval to perform study and recruit workers by the Study Director, and PFI.
6) Qualify test sites and hold informational meetings with workers at qualified ites by the Study Director and PFI.
7) Enroll worker participants through the formal informed consent process by the Study Director and PFI.
8) Perform monitoring of study participants at each site by the PFI and Field Team.

Site Selection

Criteria set as prerequisites for inclusion of a test site in this study were seed type (canola or corn), active ingredients used (imidacloprid or clothianidin and carbathiin or metalaxyl), and sufficient volume to allow for multiple days of monitoring at each site.

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This study provided an ideal opportunity for randomization of test facilities in that the entire universe of test facilities meeting the qualification requirements could be identified through BCS sales records. BCS already had existing professional relationships with the management of the potential test sites allowing effective communication of the study goals and benefits. The timing for the study was near the end of the seasonal rush for the seed types involved, providing an experienced work force comfortable with their job functions. Additionally, management and workers alike were interested in participation as it would allow them a means to evaluate their safety and chemical hygiene practices.

Upon initial qualification screening of potential test facilities it was determined that GAUCHO® would likely not be used in sufficient quantities to be included in the study. The Study Director provided the Principal Field Investigator (PFI) with a list of all facilities known to have purchased PROSPER™ FL in Canada or PONCHO® 600 FS plus ALLEGIANCE® FL or VITAVAX®-34 in the US. These formulated products are registered on canola and corn, and either alone or in the combination noted, provide the active ingredients desired for the study.

For canola treatment in Canada, the PFI reviewed the list of facilities which had purchased PROSPER™ FL, removed two BCS owned facilities ineligible for consideration, and randomly ordered the remaining facilities. Due to the limited number of commercial treatment facilities for canola, the randomized list consisted of 12 facilities. The first five facilities in random order were then contacted and asked about their plans for treating canola seed with one or more of the desired active ingredients in the time period of the study. Site visits with management personnel responsible for seed treating activities at the first three facilities that were determined to meet study criteria were then scheduled.

The Study Director and Principal Field Investigator visited with management personnel responsible for three facilities. The purpose of the study was described, the voluntary nature of facility and worker participation explained, and the process for recruiting volunteer workers thoroughly reviewed. Management of two of the facilities agreed to allow the study to be conducted if workers expressed an interest. Management of the third facility described treating plans far smaller than expected, sporadic runs of the desired seed and active ingredients which would not allow conduct of the study according to the approved protocol. The Study Director determined that sufficient MUs could be measured at the first two canola treating facilities in Canada, so they were included in the study .

For corn treatment in the United States, the Study Director provided list of facilities purchasing PONCHO® 600 FS, ALLEGIANCE® FL or VITAVAX®-34 contained in excess of 5,000 entries, including duplicate entries for facilities with multiple purchase and/or product histories. As the study protocol required several consecutive days of treat, bag and sew activities, a purchase volume threshold was applied by the Study Director to ensure facilities in the selection pool would be treating sufficient amounts of seed to allow collection of all samples. The list of facilities which previously purchased in excess of the purchase volume threshold contained 110 entries. This list was then randomized by the PFI, and the first ten facilities targeted for further evaluation. As these facilities were individual plant locations of large seed companies, employees of BCS familiar with the seed companies were contacted to identify appropriate management personnel within the seed companies. Initial contact with appropriate management personnel of the seed companies was then initiated by BCS by telephone, email and letter. Follow-up conference calls then occurred between study personnel and appropriate management personnel representing random order facilities. The conference calls yielded permission to talk with plant personnel at the three facilities which met study criteria for equipment, seed type and active ingredients. The PFI then visited and began recruitment at all three facilities.

Worker Recruitment

An IIRB approved recruiting flyer was posted at each of the five facilities along with a letter from facility management. The recruiting flyer indicated that BCS was seeking volunteers for a research study, listed eligibility criteria, gave a brief synopsis of participation, provided contact information for the PFI, and stated an informational session would be held at the facility in the near future. The management letter informed workers that BCS had been given permission to provide information about a potential research study at the facility, and visit with workers interested in learning more about the study. The letter stated participation in the study was completely voluntary and would not benefit the company, indicated management was not encouraging or discouraging participation, and noted a worker's decision to participate, not participate or withdraw would have no impact on employment or pay.

The Study Director and/or the PFI visited each of the five facilities and made a brief presentation to workers who, by attending a voluntary informational session, indicated they wanted more information about the study. The presentation reiterated information contained on the recruiting flyer, and displayed equipment and techniques to be used for sample collection. At the end of each presentation, workers were informed further information and answers to any questions they might have would be available in private meetings with the Study Director or PFI. Each worker was then paid $20 cash in local currency for attending the meeting.

Informed Consent Process

Private meetings with each individual interested in participating in the study subsequently occurred with a number of workers at each site. In each meeting, the Study Director or PFI further explained the study, reviewed eligibility criteria, discussed the concept of informed consent and specific requirements applicable to this study, provided and reviewed the Informed Consent Form and the product label(s), answered questions posed by the worker, and asked questions of the worker to confirm understanding of the voluntary nature of participation. Workers who indicated a desire to participate in the study were enrolled, provided a copy of their signed Informed Consent Form and signed Experimental Subject's Bill of Rights, a copy of the posted management letter, and reminded they could ask additional questions, or change their mind at any time without penalty.

Enrolled workers indicated they met all of the following eligibility criteria:

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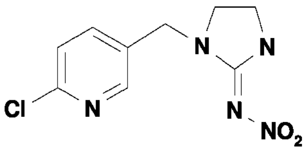
1. Worker was freely willing to participate and be observed and photographed.
2. Worker was employed by a commercial seed treating facility.
3. Worker performed mixing/treating of pesticide products for seed treatment, bagging/sewing/stacking of treated seed, or clean-out of seed treatment equipment, within the past year.
4. Worker considered himself to be in good general health, with no medical conditions that could impact his ability to participate in the study.
5. Worker performs pesticide handling tasks in conformance with product labeling applicable to seed treatment.
6. Worker anticipated working a full day on the day of exposure monitoring.
7. Worker understood English, French or Spanish.
8. Worker was not employed by Bayer CropScience, it's agents or contractees.

A total of twenty eight workers were enrolled in this study. Five workers were enrolled at Location 1 in Saskatchewan, including one who decided to withdraw prior to experimental start, and another who asked to participate after exposure monitoring commenced at the facility. Five workers were enrolled at Location 2 in Alberta. Three workers were enrolled at Location 3 in Illinois. Eleven workers were enrolled at Location 4 in Minnesota, including three whose subsequent work schedules did not allow participation in the study. Four workers were enrolled at Location 5 in Michigan.

If at any point during the worker recruitment and informed consent process either facility management or the workers decided to withdraw from the study, the Study Director and PFI would have to return to the initial randomized list and begin the entire process again with the next facility.

Table I. Structures, names, and CAS registry numbers of the active ingredients used in the study

Compound	**Chemical Structure**
	
Trade Name	GAUCHO 480F®
A.I. Chemical Name	Imidacloprid

Continued on next page.

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Table I. (Continued). Structures, names, and CAS registry numbers of the active ingredients used in the study

IUPAC Name	1-(6-Chloro-3-pyridinyl)methyl-*N*-nitroimidazolidin-2-ylideneamine
CAS Name	1-[(6-Chloro-3-pyridinyl)methyl]-*N*-nitro-2-imidazolidinimine
CAS Number of a.i.	138261-41-3

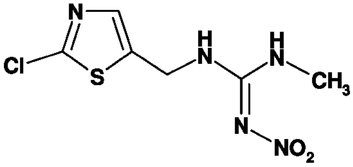
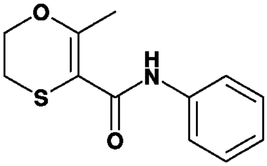
Compound	**Chemical Structure** 
Trade Name	Clothianidin
A.I. Chemical Name	TI 435
IUPAC Name	(*E*)-*N*-[(2-Chloro-1,3-thiazol-5-yl)methyl]-*N*-methyl[oxido(oxo)hydrazono] methanediamine **or** *N*-[(2-Chloro-1,3-thiazol-5-yl)methyl]-*N*-{(*E*)-(methylamino) [oxido(oxo)hydrazono]methyl}amine
CAS Name	[*C(E)*]-*N*-[(2-Chloro-5-thiazolyl)methyl]-*N′*-methyl-*N″*-nitroguanidine
CAS Number of a.i.	210880-92-5
Compound	**Chemical Structure** 
Trade Name	PROSPER™ FL
A.I. Chemical Name	Carboxin, Carbathiin
IUPAC Name	5,6-Dihydro-2-methyl-1,4-oxathi-in-3-carboxanilide
CAS Name	5,6-Dihydro-2-methyl-*N*-phenyl-1,4-oxathiin-3-carboxamide
CAS Number of a.i.	5234-68-4

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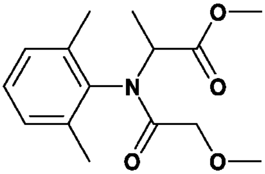
Table I. (Continued). Structures, names, and CAS registry numbers of the active ingredients used in the study

Compound	**Chemical Structure** 
Trade Name	PROSPER™ FL
A.I. Chemical Name	Metalaxyl
IUPAC Name	Methyl *N*-(2-methoxyacetyl)-*N*-(2,6-xylyl)-***DL***-alaninate
CAS Name	Methyl *N*-(2,6-dimethylphenyl)-*N*-(2-methoxyacetyl)alanine
CAS Number of a.i.	57837-19-1

Field Logistics

Study Team

For data collection, each test location was visited by a study team consisting of six to seven researchers: the Study Director, PFI, a chemist to perform field fortifications, two to three observers, an emergency medical technician, and a quality assurance auditor. The Study Director, PFI, and chemist generally remained away from operations that might result in contamination of study samples and performed all operations associated with sampling including: dressing the workers, performing hand washes and face/neck wipes, performing field fortifications, and sample collection and processing. The observers remained with their assigned worker(s) throughout the day and recorded all work activities, logged the amounts of seed and test chemicals handled, and noted any actions or conditions that may have influenced the exposure of the workers to the test substances.

Work Activities

Each Monitoring Unit (MU) consisted of a single worker performing their normal work activities, either as a treater, bagger/sewer/stacker, or as cleanout personnel. Workers experienced at the activity being performed were monitored performing their regular work activities. A summary of worker information (location, job function, date(s) monitored, age, height, weight, and work experience) was recorded. All workers wore an inner dosimeter under a single layer of clean clothing consisting of shoes plus socks, long-sleeved shirt and long pants. At the end of each work day, all monitored workers were given $80 to compensate them for the inconvenience of participating in the study. The compensation amounts were consistent with those previously determined by both Regulatory Agencies (PMRA, USEPA) and the HSRB to be appropriate. Since participation was totally voluntary, not all job functions were monitored at each test location. Treaters were monitored at locations 2, 3, 4, and 5. Bagger/sewer/stackers were monitored at all five locations. Cleanout personnel were monitored at locations 1, 3, 4, and 5.

Typical workflow involved one treater who prepared treatment solutions/ suspensions, operated equipment which transferred raw seed into the treater, operated the treating equipment, and monitored coverage of treated seed, amount of product used, and amount of raw seed treated. When using manual bagging equipment, two to three workers operated the bag and sew equipment, placing bags on fill spouts, arranging bags and positioning tags as bags entered the sewing head. Two workers typically were engaged in manually stacking bags of treated seed on pallets. One or two workers operated forklifts, removing completed pallets from the stacker. Workers performing sew/bag/stack activities typically rotate jobs during the work day. All bagging and sewing (but not stacking) using fully automated equipment was performed by a single worker.

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Sample Collection and Handling

In each trial, four types of samples were collected to monitor potential worker exposure. Inner dosimeters were used to measure potential dermal exposure through clothing. Hand washes were performed to evaluate potential dermal exposure to the hands. Face/neck wipes were used to evaluate potential dermal exposure to the face and neck. Airborne concentrations of the test chemicals in the worker's breathing zone were monitored utilizing an OVS tube sample collector. Procedures for collecting each type of sample are outlined below.

Inner Dosimeters

The inner dosimeters, worn directly under the outer clothing, consisted of a one-piece, white, 100% cotton long-underwear union suit purchased from Carolina Apparel Group, Inc. in Wadesboro, NC. Inner dosimeters were laundered prior to use to minimize any potential contamination.

At the end of each monitoring period, the inner dosimeter was removed in a clean dressing area, taking care to ensure outer shirt and pants did not contaminate the inner dosimeter. The field investigator(s) assisting the worker put on a new pair of latex gloves prior to removal of the inner dosimeters. Buttons were removed from the inner dosimeter, and the dosimeter cut into six sections. Scissors used to cut the dosimeter sections were rinsed with acetone between MUs. Dosimeter sections included:

Left/Right Upper arms combined (elbow to shoulder seam)
Left/Right Lower arms combined (elbow to cuff)
Left/Right Upper legs combined (waist to knee)
Left/Right Lower legs combined (knees to cuff)
Torso - front (above the waist)

Torso - back (above the waist)

All dosimeter samples were wrapped in aluminum foil prior to placement in plastic re-sealable bags labeled with the study number, sample number, and collection date. Samples were then placed into a freezer and stored frozen until analyses.

Hand Washes

The worker placed both hands over a stainless steel bowl and washed them as a researcher poured 400 mL of an aqueous solution of 0.01% v/v Aerosol OT (AOT) over the hands. The worker then scrubbed his hands together in the wash solution for approximately 30 seconds. The worker then removed his hands from the solution and held them over the bowl while the field investigator poured a final 100 mL AOT rinse over the palm and back surfaces of the hand. The entire 500-mL sample was then decanted into a labeled glass jar with Teflon® -lined lid, placed into a plastic re-sealable bag, and then into a field cooler. At the end of the monitoring period, aliquots of all hand wash samples were amended with an internal standard solution containing a known amount of an isotopically enriched stable-labeled analog of each analyte. The solutions were well mixed and then extracted using solid phase extraction (SPE) cartridges. The SPE cartridges were placed in plastic re-sealable bags labeled with the study number, sample number, and collection date. Samples were then placed into a freezer and stored frozen until analyses.

A hand wash was conducted prior to the work day to familiarize the worker with the procedure and ensure no contamination was brought into the study. This wash was discarded. During the monitoring period, hand washes were performed at any bathroom and lunch break. A hand wash was also performed at the end of the monitoring period. The number of hand washes per MU ranged from one to six. Each hand wash was a separate sample.

Face/Neck Wipes

A 4 x 4 inch, 8 ply, 100% cotton gauze pad (manufactured by Kendall Curity, Mansfield, MA) was moistened with 4 mL of 0.01% (V/V) Aerosol® OT (AOT) solution. The pad was then wiped across the face, front and back of the neck of the worker by a field investigator wearing clean latex gloves. The pad was then placed on a piece of aluminum foil. The process was repeated with a second pad, combining both pads on the same foil. The foil was then folded, placed in a plastic re-sealable bags labeled with the study number, sample number, and collection date, and the bag placed in a freezer.

A face/neck wipe was conducted prior to the work day to ensure no contamination was brought into the study. This wipe was discarded. Additional face/neck wipes were then performed whenever the worker had something to eat, typically at morning break, lunch break, and afternoon break. A final face/neck

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wipe was collected at the end of the monitoring period. All wipes were placed in the same pre-labeled re-sealable plastic bag, for a total of one face/neck wipe sample per replicate. Face/neck wipe samples were stored frozen until analyses.

Air Sampling

Airborne concentration of active ingredients in the worker's breathing zone were monitored with an OSHA Versatile Sampler (OVS) tube connected by Tygon® tubing to a uniquely numbered SKC Airchek 2000, or SKC PCXR8 personal air sampling pump. The pumps were calibrated before each MU to operate at a flow rate of approximately 2.0 liters per minute (LPM). The OVS tube consisted of a glass fiber filter at the air inlet, followed by two sections of XAD-2 (consisting of 270 and 140 mg separated by a polyurethane plug) housed in a 13 mm diameter glass tube. The OVS tubes were manufactured by SKC, Inc. The OVS tube was held in a plastic tube holder and clipped to the worker's outer shirt collar with the intake facing downward.

The air sampling pumps operated throughout the monitoring period including breaks and lunch, except for workers opting to take an off-site meal break; in those cases, the pump was removed while the worker was off-site. At the end of each MU, the Tygon® tube, pump and OVS tube were removed from the worker and the airflow rate was measured. The OVS tube was then disconnected from the tubing, capped at both ends, wrapped in bubble wrap, placed into a re-sealable plastic bag, and then placed in a freezer.

Field Fortifications

One MU at each location included collection of field fortification samples in conjunction with incurred-residue sample collection. These samples were used to verify the integrity of residue samples throughout collection, storage, and shipment. Incurred residue values were adjusted based on the recovery of residues from the appropriate field fortification samples. The field fortification samples were stored and shipped under the same conditions used for the incurred-residue samples.

Inner dosimeter fortification samples (3 low level at 5.00 µg of each test substance, 3 mid level at 1000 µg of each test substance, and 3 high level at 5000 µg of each test substance) were generated at each location. Control dosimeter pieces were amended with the appropriate amounts of a mixture of analytical standards of the four test substances in the field at the time of sample collection. The spiked samples were treated and aged, under a single layer of cloth to simulate the outer garment of the mixer/treater/planter, for approximately the same duration, and under similar conditions, as the incurred-residue samples. The field fortification samples were amended and maintained in an area free from possible contamination from the test substance. Following the aging period, these samples were immediately stored in a freezer.

Control hand wash solution was amended with known amounts of each test substance (3 low level at 5.00 µg of each test substance, 3 mid level at 1000 µg of

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each test substance, and 3 high level at 5000 µg of each test substance) in the field at the time of sample collection and the solutions were treated in the same manner as incurred-residue samples (addition of internal standard solution and preparation of triplicate SPE cartridges).

Control gauze pads were amended with known amounts of of each test substance (3 low level at 5.00 µg of each test substance, 3 mid level at 100 µg of each test substance, and 3 high level at 2500 µg of each test substance) in the field at the time of sample collection. The fortified samples were treated and immediately stored frozen.

OVS tubes fortified with a mixture of of each test substance analytical reference standard at three levels (3 low level at 0.050 µg of each test substance, 3 mid level at 1.00 µg of each test substance, and 3 high level at 50.0 µg of each test substance) were shipped frozen to the field trial sites. At the time of field fortification sample generation, the fortified OVS tubes were allowed to warm to ambient temperature and then were attached to a sampling pump. In an area located away from the possibility for exposure, air was drawn through the fortified OVS tubes during the entire exposure period at the flow rate used for the study. At the conclusion of the ageing period, the fortified samples were stored frozen for shipment.

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Lessons Learned

The main lesson learned from this study is that when conducting an observational study, the study team has absolutely NO CONTROL. Scheduling, either scheduling the run being monitored or individual activities within the run, is completely at the discretion of facility management. The study team will need to adapt to variation in work timing (multiple shifts, odd hours, etc.) and work practices that are unique to each test location.

The logistics of pre-locating personnel and equipment in anticipation of constantly changing production schedules is challenging. There were high travel costs arising from the short lead times provided by facility management based on their changing production priorities. Prior planning was especially important in transporting laboratory equipment, samples, supplies, and personnel across international borders.

The use of human subjects, even in a totally observational study, remains subject to a check-box approach to study design by some regulatory authorities. An example of this was the requirement to log temperatures, calculate heat indices, and have an on-site medical professional to monitor the test subjects for heat stress. While this requirement is appropriate for studies performed in mid-summer on farm sites, it was probably not needed for seed bagging in a warehouse in northern Sasketchewan in mid-March with an outside temperature of -40°C.

Conclusion

The main advantage of observational studies is that they do not require review or protocol approval by the EPA Human Studies Review Board (HSRB). However, all of the requirements of US EPA 40 CFR Part 26 Protection of Human Subjects and 40 CFR Part 160 FIFRA Good Laboratory Practice Standards still apply. It is best to obtain concurrance that the study is, in fact, observational in nature from the appropriate authorities prior to initiation. Observational studies also appear to pick up work habits that are not as likely to appear in an intentional dosing study.

Test location selection and test subject recruitment should be made a straightforward and transparent as possible to aviod bias in the study results. The aid of an institutional review board in reviewing this process is strongly advised. To offset the high costs of these studies, try to have multiple opportunities for observation at each test location.

The use of observational studies should be considered on a study-by-study basis where the additional trouble and expense may be justified.

The choice of performing this study as an observational study, while generating a unique set of challenges, allowed for the measurement of exposures under actual production conditions.

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Chapter 8

Exposure to Pesticides in the Greenhouse: A New Modeling Approach in Europe

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Operator exposure studies sponsored by the European Crop Protection Association (ECPA) were performed in greenhouses in 2002-2006 within a larger experimental exposure monitoring program in Southern Europe. Results are compiled in a database and a proposal for exposure modelling is made. The data base contains information from 7 applicator studies undertaking hand-held spray application using a spray gun/lance connected to a stationary tank and 10 mixing/loading studies using solid or liquid formulations. Studies were conducted in Spain, Italy and Greece. Both inhalation and dermal exposure (potential and actual) were measured via a whole body passive dosimetry technique. Analysis of work practices and exposure data identified exposure scenarios for high and low crops and further sub-scenarios in both cropping systems for negligible/no contact with treated foliage and direct contact with treated foliage. The developed data package including a model for use in regulatory exposure assessment is in a commenting period with the European Member States, EU Commission and the European Food Safety Authority (EFSA).

Introduction

The registration of pesticides in Europe under Regulation (EC) No 1107/2009 of the European Parliament (effective as of 14 June 2011) and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directive 91/414/EEC requires the use of models

In Non-Dietary Human Exposure and Risk Assessment; Krolski, M., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2010.

in the Annex I and III risk assessment process. Operator exposure during the mixing/loading and application of pesticides must be evaluated taking into account normal work clothing (no PPE) and additional Personnel Protective Equipment (with PPE).

Data gaps were identified within the existing European models (UK Predictive Operator Exposure Model (UK POEM) (*1*), German BBA model (*2*) and EUROPOEM (*3*)) for hand-held application to crops, particularly in Southern Europe. An additional data gap was identified for the mixing and loading of solid formulations and this was addressed by the monitoring of the mixing and loading of powder/granule formulations. To address these data gaps, 14 operator exposure studies, 7 in greenhouses, were conducted according to OECD Guidance (*4*). All studies were in full compliance with GLP requirements.

The studies were sponsored and monitored by ECPA. The scope of the project was to develop a data package that supports a modelling approach (definition of model, calculation spreadsheet, overview document, field reports and assessment reports).

The Study Programme

In order to focus on the most representative and likely worst case exposure conditions for operators handling pesticides in greenhouses a detailed understanding and description of use conditions was developed before the start of the studies e.g. application technique, area treated, duration of application, the working clothing and PPE in use and their relevance to the greenhouse situation. An initiative undertaken by ECPA to look at agronomic practices in Southern Europe (Safe Use Initiative) was also a source of information on cropping practices and work practices.

The term greenhouse comprises different protection structures such as low tunnels, walk-in tunnels, shade houses, plastic greenhouses or glasshouses. Also, various application techniques exist for pesticide application in greenhouses e.g. drip application, fogging or dusting. Mainly, however, spray applications are performed either manually (hand pulled trolley, spray rigs, spray lance, spray gun) or supported by tractors (inter alia self-propelled sprayers, tractor airblast sprayers (canon sprayer)).

The use of a large spray tank, either stationary, or a tractor tank with hose connected to a hand-held spray gun was identified as the most important and common in greenhouses across Southern Europe. In addition, the impact of cropping techniques (e.g. intensity of planting) was considered in relation to potential sub-setting of the data for inclusion in a model.

The operators wore polyester/cotton standard working coveralls, but in certain cropping scenarios, rain suit coveralls/trousers are commonly used. Nitrile gloves and sturdy footwear were also standard equipment.

The greenhouse studies were conducted during the period 2002 to 2006. The studies focussed on the exposure resulting from application, but additional information on the mixing and loading associated with spray tanks was also obtained. Additional studies conducted on field crops as part of the overall project also generated data for mixing/loading liquid and solid formulations into large tanks. These data are included in the model in the absence of mixing/loading liquid formulation data in the greenhouse studies. Study details are summarised in Table 1.

The exposure was determined using standardised passive dosimetry methodology. This entailed the use of inner and outer dosimeters for body exposure, protective gloves and hand washes for hand exposure, face and neck washes for head exposure. Inhalation exposure was monitored using a suitable collection device located in the breathing zone to collect the inhalable fraction of airborne particles.

Data was generated for the mixing/loading phase and separately for the application phase. These studies were conducted according to OECD Guidance for the conduct of studies of occupational exposure to pesticides during agricultural applications (*4*) and were GLP compliant for the field, analytical and report phases, including assessment reports. The studies were monitored by ECPA and conducted using internationally recognised contract research organisations.

In total 216 operators (102 applicators and 114 mixer/loaders) were monitored during a representative working day. Some study details are presented in Table 2.

Table 1. Overall Summary of Studies used for the Greenhouse Model

EOEM Study ID-*	*Country*	*Region*	*Crop*	*No of Operators*	
				Mix/Load	*Applic.*
Greenhouse studies					
02	Spain	Almeria	Peppers	10	32
03	Spain	Almeria	Cucumber	10	10
10	Italy	Tuscany/Vento	Pot Plants	10	10
12	Spain	Murcia/Alicante	Cucumber	10	10
13	Spain	Murcia/Alicante	Tomato	10	10
14	Italy	Sicily	Melon	20	20
15	Italy	Sicily	Melon	-	10
Additional mixer/loader field studies					
04	Spain	Valencia	-	10	-
05	Greece	Macedonia	-	12	-
07	Spain	Valencia	-	10	-
08	Spain	Andalucia	-	12	-

* EOEM = ECPA Operator Exposure Monitoring.

Table 2. Selection of study details

Area treated (ha/day)	*Actual working time (mins)*		*Crops*	*Crop height (m)*	*Row distance (m)*	*Appl. volume (L/ha)*
	Appl.	*Mix/ load*				
0.1-1.1	17-202	3-40	Cucumber	1.3-2.5	1.3-3.0	350-2400
			Tomato	1.6-2.4	1.0-2.5	
			Pepper	1.1-2.1	0.8*-2.0	
			Pot plants	0.08-0.30	-	
			Melon	0.5 (<1)	-	

* in some cases 0.2 m distance between foliage.

The height of cucumber, tomato and pepper crops was above 1 m. They were considered representative for high crops. On the other hand, pot plants grown on soil with heights of 7.5-30 cm and melons with a height of usually below 0.5 m (only rarely reaching up to 1 m) were considered as low crops. Inter-row distance for the crops varied considerably from 3 m down to 0.8 m (in some cases distance between foliage was only 0.2m). No inter-row spacing existed in melon and pot plants. The application volume ranged from 350-2400 L/ha.

Separate field phase/analytical reports were compiled that contain:

- detailed description of the individual applications in terms of location, application (area treated, duration, equipment, clothing), tasks monitored, experimental procedures (dosimetry, hand wash, face wipes, field fortifications);
- detailed description of the analytical method and procedures for the dosimetry, and laboratory and field recovery samples;
- raw data for individual sample analyses and summarised as mass (mg or µg) of exposure for each individual operator.

Additional assessment reports were generated for each study to summarise dermal and inhalation exposure, to assess the limit of quantification and recoveries and any impact on the results, to assess the efficiency of the coverall and gloves protection and to perform a statistical analysis of the data including arithmetic/geometric mean, standard deviation, a range of percentiles from 25th to 95th and identification of potential outliers. The exposure results for each individual operator were calculated in terms of µg/person, and in a range of normalisation units e.g. µg/kg active substance (a.s.) handled, mL spray/h, and µg/kg bw (bw - body weight).

The Greenhouse Model

The normalised data generated in each individual assessment report were compiled into a single spreadsheet. The number and quality of the data permitted the compilation of a model – the 'Greenhouse Model'. The model enables the calculation of exposure of unprotected and protected operators. It is a Microsoft Excel®-based spread sheet in which all values are linked to work sheets containing original source data. Default exposure values are based on 75th percentile mg/kg a.s. The calculation of the geometric mean is also possible.

Four exposure scenarios were defined: high crops vs. low crops and standard vs. intensive contact. Where no data were generated standardised exposure mitigation factors for use of PPE were generated i.e. 'actual' exposure (measured exposure considering one layer of clothing) was preferred to 'potential' exposure combined with a mitigation factor. A body weight default value of 70 kg is used, based on the 25th percentile of actual body weight data contained within the studies supporting the model.

The use of the model is facilitated for specific compound evaluation by applying the normalised value (specific exposure calculated as mg/kg active substance (a.s.) handled) for the exposure in the following algorithm which allows the absorbed dose to be calculated:

$$\boxed{AD = [(E \times A \times R) \times AB] / BW}$$

Where:

AD - Absorbed dose (mg/kg bw/day)
E - Specific exposure (mg/kg a.s. handled)
A - Application rate (kg a.s./ha)
R - Work rate (ha/day)
AB - Absorption (dermal and inhalation, %)
BW - Body weight (70 kg/person)

Subsets of Exposure Data

Analysis of the exposure data confirmed the field observations that two cropping scenarios exist in terms of exposure to operators and that within these scenarios, two further sub-sets exist relating to the degree of contact between the worker and the crop.

Cropping scenarios:

- High crops
  (cucumber, tomato, pepper, etc.) i.e. > 1 metre;
- Low crops
  (melon, pot plants on soil, etc.) i.e. < 1 metre.

In Non-Dietary Human Exposure and Risk Assessment; Krolski, M., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2010.

Exposure scenarios for each cropping scenario:

- Standard scenario
  i.e. wide rows, exposure via contact with the spray, no contact with treated foliage;
- Intensive crop contact scenario
  i.e. narrow or no rows, additional exposure via direct contact with treated foliage which cannot be avoided.

To ensure consistency with the requirements of Annex I and III procedures under Directive 91/414/EEC and Regulation (EC) No 1107/2009, provision is made for the calculation of exposure for both the unprotected and protected operator during mixing, loading and application for all scenarios.

The notion 'unprotected' is still inconsistently used in the current European exposure models. The unprotected operator is considered to wear no PPE;, however, there is no consensus on the level of clothing that this operator wears (body either fully covered or only partly covered by clothing). With the development of the greenhouse model a contribution is made to a harmonization of the definition of an 'unprotected' and a 'protected' operator according to Good Agricultural Practice:

An unprotected operator is considered to wear a minimum level of clothing (standard clothing) consisting of shoes, socks and one single layer of clothing covering the body (excluding head and hands). This is considered to be a conservative assumption in risk assessment as underwear which is typically worn in addition is not taken into consideration for exposure modelling. An option is offered in a separate model spreadsheet to calculate exposure of operators who do not follow minimum working clothing recommendations i.e. wearing only a T-shirt and shorts.

In addition to the minimum clothing scenario, the protected operator may wear an additional level of clothing/equipment i.e. protection which is certified as PPE according to EU standards.

The exposure scenarios of the model are thus compiled from potential dermal exposure values (sum of residues on outer and inner dosimeters), actual dermal exposure values (residues on inner dosimeters) and inhalation exposure values. Where no actual dermal exposure values were measured the potential exposure values are used in conjunction with a default protection factor to estimate the actual exposure.

The exposure of an unprotected operator during mixing and loading is compiled from potential dermal exposure values for the hands and from inhalation exposure values. As body and head exposure during mixing/loading is considered to be negligible this was not separately measured and therefore not included in the mixing/loading scenario. Where operators were not monitored separately during mixing/loading and application the portion of exposure arising from mixing and loading is included in the body exposure during application.

The exposure of the unprotected operator during application is calculated from potential dermal exposure values for the head and hands, actual dermal exposure of the body (because a standard working coverall was worn) and potential inhalation exposure. The scenario for operators wearing T-shirt & Shorts comprises potential exposure values for inhalation, head, hands, ½ upper arm, forearm and legs to account for dermal exposure of body parts not covered by clothing as well as actual exposure data for trunk and ½ upper arm to account for dermal exposure of body parts with single layer clothing.

Protected operators additionally wear PPE. Exposure results are based either on measured or calculated data. Where no measured data is available the exposure reduction coefficients for the use of various items of personal protective equipment or clothing are based mainly upon those used in the German BBA model (*2*).

For the protected operator during mixing and loading, actual dermal exposure values for the hands and potential inhalation exposure values along with the use of exposure reduction coefficients (protection factors) for the use of respiratory protective equipment are utilized for all scenarios.

For the protected operator during application, different permutations of potential dermal exposure values for the head and potential and actual dermal exposure values for the body (upper and lower or combined), actual hand exposure values and potential inhalation exposure values are utilized, along with the use of exposure reduction coefficients (protection factors) for the use of specified personal protective equipment and clothing.

Mixing/Loading Phase: Summary of the Normalised Exposure Data

Exposure was monitored for mixing/loading liquid and solid formulations into a large spray tank. The data set for liquids consists of exposure data from operators mixing/loading a suspension concentrate (SC formulation). Wettable powder (WP formulation) and water dispersible granules (WG formulation) were representative solid formulations. Photographs for typical working conditions during mixing/loading are presented in Figures 1, 2, and 3 .

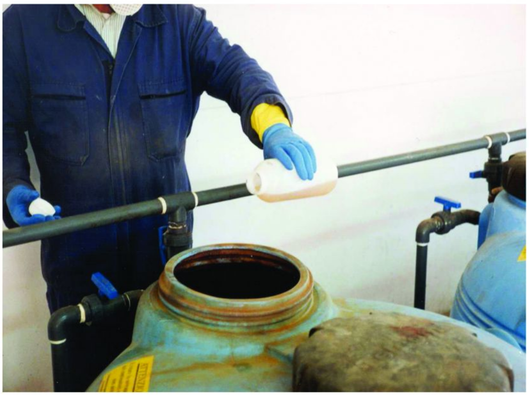

Figure 1. Mixing/loading liquids

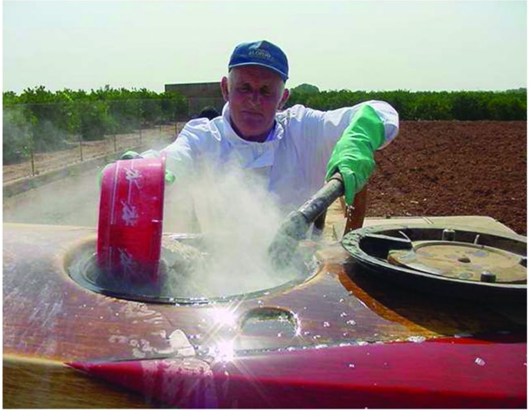

Figure 2. Mixing/loading solids (WP)

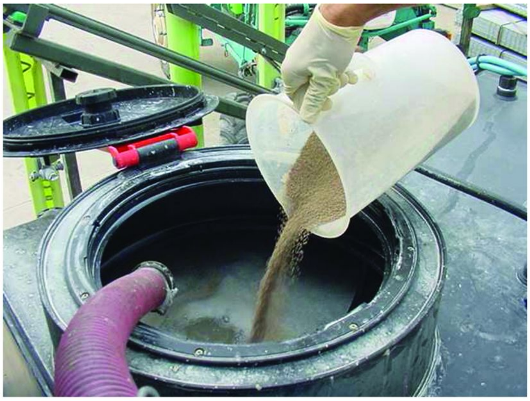

Figure 3. Mixing/loading solids (WG)

Dermal hand exposure and inhalation exposure were measured in all but one of the greenhouse studies comprising the applicator data set. These data form the core data set for mixing and loading. A number of other relevant EOEM outdoor field studies have been incorporated into the model because they involved mixing and loading large spray tanks that are essentially the same as those used for preparing spray mixtures for application in greenhouses. The respective exposure values for unprotected and protected mixer-loaders are shown in Table 3.

Table 3. Operator exposure (75th perc.) during mixing/loading for greenhouse applications

Exposure Route	*Unprotected (mg/kg a.s. handled)*	*Protected (mg/kg a.s. handled)*
	Liquids	
Hands	2.01	0.0223
Inhalation	0.00005	0.000004
	Wettable Powder (WP)	
Hands	29.0	0.316
Inhalation	0.241	0.0193
	Water dispersible Granule (WG)	
Hands	2.29	0.0297
Inhalation	0.0137	0.0011

Application Phase: Summary of the Normalised Exposure Data

High Crops – Standard Scenario

Photograph for typical working condition is presented in Figure 4.

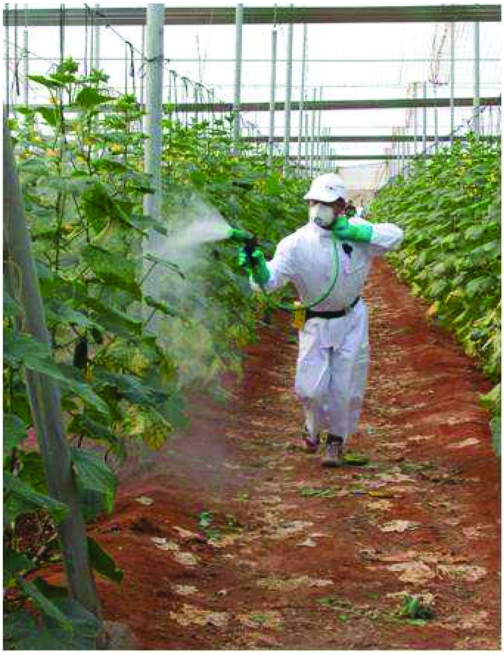

Figure 4. High Crops – Standard Scenario

This scenario is characterized by wide rows, exposure via spray, no contact with treated foliage. Analysis of the exposure distributions had shown that the data for the application phase were similar. Therefore, the data of the three studies are combined into one data sub-set. Data are available for both unprotected and protected operator exposure predictions (Table 4).

Table 4. Operator exposure (75th perc.) during application in High Crops - Standard Scenario

Exposure Route	***mg/kg a.s. handled***	***mg/kg a.s. handled***
	Unprotected[1]	***Protected***[2]
Body	17.1	17.1
Head	0.806	0.322
Hands	25.2	0.022
Inhalation	0.678	0.054

[1] Example for an operator wearing an uncertified working coverall (cotton/polyester) working with bare hands and without PPE. [2] Example for a range of additional PPE permutations: hat (head), gloves (hands), FFP2 respirator (inhalation/head). An example calculation for additional body protection is not presented as it seems unlikely that operators will use impervious coveralls in the standard high crop scenario (wide rows).

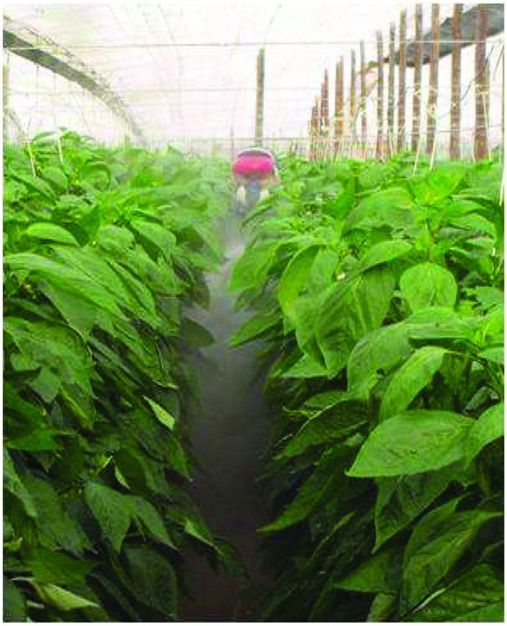

Figure 5. High Crops – Intensive Contact Scenario

In Non-Dietary Human Exposure and Risk Assessment; Krolski, M., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2010.

High Crops – Intensive Contact Scenario

Photograph for typical working conditions is presented in Figure 5.

This scenario is characterized by narrow rows resulting in additional exposure via direct contact with treated foliage. The data involving application to peppers, are substantially different to those of the high crop – standard scenario and thus form a unique high crop intensive exposure scenario. Under these working conditions, operators typically wear impervious clothing e.g. rain suits to protect from drenching of clothing. This is in accordance with Good Agricultural Practice. Therefore, calculation is only available for the protected operator (Table 5) wearing impervious clothing and protective gloves. A safety phrase must always be incorporated on product labels for this scenario to ensure that contact with treated crop must be avoided by use of spray tight protective clothing (Cat. III, type 4), or use of engineering controls. [Contact with treated foliage can be avoided by changing the application modus operandi, e.g. walking backwards when spraying, or engineering methods (e.g. use of pulled trolley). The intensive crop contact scenario is modified to standard. In this case a standard working garment can be worn instead of certified spray tight protective clothing.] The representative data for the protected operator are therefore the potential inhalation, and potential dermal exposure values for the head and actual dermal exposure data for the hands and body, the latter derived from the measurements taken beneath the rain suits worn by the operators. Data from non certified rain suits are taken as conservative surrogates for certified spray tight protective clothing (Cat. III, type 4) which have proven in additional trials to be more protective than uncertified rain suits.

Table 5. Operator exposure (75th perc.) during application in High Crops – Intensive Contact Scenario

Exposure Route	***mg/kg a.s. handled***
	Protected[1]
Body	2.17
Head	0.043
Hands	1.05
Inhalation	0.066

[1] Example from a range of clothing and PPE permutations: mandatory protective clothing and gloves and an example for head and for respiratory protection. Calculations include spray tight clothing (body), gloves (hands), hood and face shield (head), and FFP2 respirator (inhalation/head).

Low Crops – Standard Scenario

Photograph for typical working condition is presented in Figure 6.

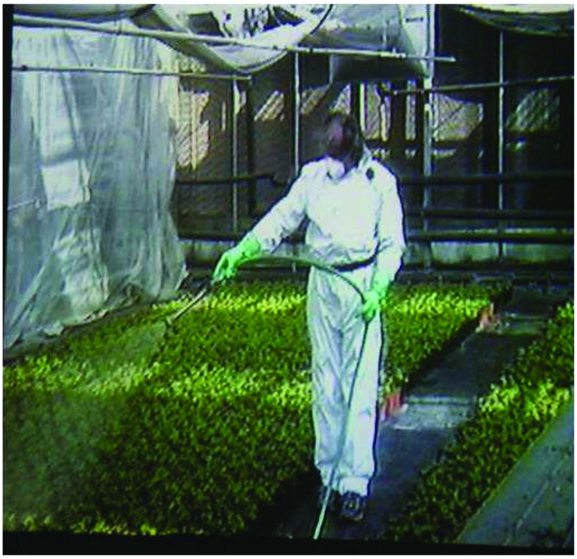

Figure 6. Low Crops – Standard Scenario

This scenario is characterized by wide rows, exposure via spray, no contact with treated foliage. This scenario and study data differ substantially from the other low crop scenario and data and therefore comprise a unique sub-set. In this scenario exposure arises predominantly from drift. Data are available for both unprotected and protected operators (Table 6). The unique feature of application in all low crops is the potential for substantial exposure of the lower part of the body, i.e. the legs. Therefore, it is important to facilitate the differential protection of this body area. The combination of data for the protected operator calculation consists of potential inhalation, potential head, and actual body and hand dermal exposure values.

Table 6. Operator exposure (75th perc.) during application in Low Crops – Standard Scenario

Exposure Route	*mg/kg a.s. handled*	*mg/kg a.s. handled*
	Unprotected[1]	*Protected*[2]
Body	0.373	0.373
Head	0.011	0.0046
Hands	5.71	0.0002
Inhalation	0.443	0.035

[1] Example for an operator wearing an uncertified working coverall (cotton/polyester) working with bare hands and without PPE. [2] Example calculation includes standard coverall (body), gloves (hands), hat (head), and FFP2 respirator (inhalation/head). An example calculation for additional body protection is not presented as it seems unlikely that operators will use impervious coveralls in the standard low crop scenario.

Low Crops – Intensive Contact Scenario

Photograph for typical working condition is presented in Figure 7.

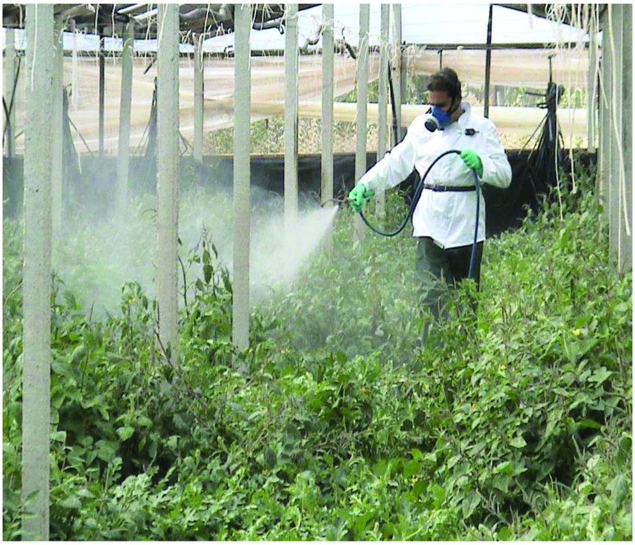

Figure 7. Low Crops – Intensive Contact Scenario

This scenario is characterized by narrow or no rows resulting in additional exposure via direct contact with treated foliage which cannot be avoided. The predominant source of exposure in this unique, intensive contact scenario is direct contact with the low growing treated crop and so involves the lower part of the body, particularly the legs. Most of the operators confirmed that they usually wear spray tight trousers and not cotton/polyester trousers for application in low growing, narrow or no row crops. Therefore, actual dermal exposure data for the lower body, involving the use of spray tight trousers, are used for the protected operator calculation. (The same rationale for rendering contact with treated crop negligible, as mentioned under the 'High Crops – Intensive Contact Scenario', also applies to this one. Modification of the application modus operandi, e.g. walking backwards when spraying, or engineering methods, can modify this scenario to standard. In this case a standard working garment can be worn instead of certified spray tight protective clothing.) These are in addition to the potential inhalation exposure values, potential dermal head exposure values and actual dermal hand exposure values (Table 7).

Table 7. Operator exposure (75th perc.) during application in Low Crops – Intensive Contact Scenario

Exposure Route	*mg/kg a.s. handled*	*mg/kg a.s. handled*
	Unprotected	*Protected*[1]
Body	305	1.28
Head	0.364	0.146
Hands	26.8	0.038
Inhalation	1.46	0.117

[1] Example calculation includes spray tight trousers (lower body), cotton jacket (upper body), gloves (hands), hat (head), and FFP2 respirator (inhalation/head).

Exposure Mitigation (Protection) Factors Used in the Model

Data were generated for the use of working coveralls and protective gloves. Where data were not generated for the use of PPE the existing default exposure mitigation values (or exposure reduction coefficients), with the exception of that for the spray tight coverall, are taken from the German Model (*2*). Values are presented in Table 8.

Table 8. Greenhouse Model Exposure Mitigation Factors

Elements of PPE	*Exposure Mitigation Factor*	
	Dermal	*Inhalation*
Headgear (hat or cap)	0.5	
Hood with faceshield	0.05	
Particle filtering mask FFP2	0.8	0.08
Half mask with combination filter A1P2	0.8	0.02
Spray tight coverall	0.005*	

* Indicative conservative value from EOEM study data

Conclusion and Outlook

ECPA has developed a data package for review by the European Member States including a model spreadsheet, an overview document (user manual), individual field reports, individual assessment reports and a presentation of the work programme and model.

The database of operator exposure data was compiled specifically to address the current data gaps for the use of plant protection products in southern European greenhouses. It consists of seven studies for mixing, loading and application in greenhouses and an additional four outdoor studies from which relevant mixing and loading data are taken. All studies meet the current OECD standards of study design and methodology and are fully compliant with the requirements of GLP.

A spreadsheet-based model has been developed from the database to enable predictive calculations of operator exposure in the greenhouse to meet the requirements of Annex I and III under Directive 91/414/EEC and Regulation (EC) No 1107/2009.

The data package including the model was initially presented by ECPA to different European Member States to receive feedback and acceptance of the model for a harmonized pan-European use. The Southern European Work Sharing Project Group took over the initiative and announcement was made at the Legislative Working Group meeting and the Standing Committee (EU Commission) as well as to the European Food and Safety Authority (EFSA). Further technical coordination resulted in a workshop in 2009 attended by stakeholders consisting of Member States and industry experts. As of the first quarter of 2010, the model is in an advanced commenting process for the implementation in the regulatory process in Europe.

Acknowledgments

This study was supported by the European Crop Protection Association (ECPA). Special gratitude is expressed to the project leader H.Felber and to the members of the ECPA expert group P.Beilstein (Syngenta), R.Brennecke (Bayer CropScience), P.Fisher (Bayer CropScience), T.Hertner (Syngenta), S.Iyengar (Bayer CropScience), P.Knowles (Syngenta), B.Krebs (Bayer CropScience), W.Maasfeld (Bayer CropScience), S.McEuen (Dupont), J.Perkins (Dow AgroSciences), M.Schneider (Syngenta), C.Schulze-Rosario (Knoell-Consult), M.Soufi (Dupont), R.Stadler (BASF), F.Stauber (BASF), L.Tilbury (Dupont), M.Urtizberea (BASF), H.Wicke (Bayer CropScience) for technical advice, steering and monitoring. Special thanks also to G. Chester (OCCUBEX RA), P.G.Pontal and I. Thouvenin (CEHTRA) for consultancy work. Collaboration with Huntingdon Life Sciences, CEMAS, Agrisearch, Inveresk Research and Agrochemex for field and lab support was greatly appreciated. Thanks to D. Pike (Consultant to Reading University Statistical Services Centre (SSC)), J.Gallagher (Reading University SSC) and Catherine Pallen (Bayer CropScience) for statistical support in this project. The author wishes to thank also the participating farmers and workers who volunteered for this project in Spain, Italy and Greece.

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3. *The Development, Maintenance, and Dissemination of a European Predictive Operator Exposure Model (EUROPOEM) Database*; AIR3 CT 93-1370, Draft Final Report; BIBRA International: Carshalton, U.K., December 1996.
4. *Guidance Document for the Conduct of Studies of Occupational Exposure to Pesticides during Agricultural Application*; OECD Environmental Health and Safety Publications; Series on Testing and Assessment No. 9; Organisation for Economic Co-Operation and Development (OECD): Paris, 1997.

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Chapter 9

Analytical Methods Developed for an Observational Worker Exposure Study Involving Multiple Target Compounds

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A study was performed by Bayer CropScience to determine the potential exposure of workers involved with commercial seed treatment systems to four active ingredients: imidacloprid (a broad-spectrum insecticide), clothianidin (a root-systemic insecticide), carboxin (a fungicide), and metalaxyl (a fungicide). Four types of samples were generated in order to measure worker exposure: dosimeter garments, face and neck wipes, hand washes, and OVS tubes. Laboratory methods were developed to measure the attained samples for the four active ingredients.

Method Summary

Dosimeter garments, face and neck wipes, hand washes, and OSHA versatile sampling (OVS) tubes were received from the seed treatment facilities and extracted. The extracts were amended with appropriate isotopically labeled internal standard solutions (IS) prior to any clean up, and after clean up the aliquots of the extracts were analyzed by liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS).

All four active ingredients were measured in one analytical run. Limits of quantitation (LOQ's) were established at 0.01 ug/sample for OVS tubes and 0.10 ug/sample for all other matrices. The linear range established on the LC/MS-MS was from 0.10 ng/mL to 15000 ng/mL, which supports the levels of fortified samples that were supplied from the field, in addition to the established LOQ's.

In Non-Dietary Human Exposure and Risk Assessment; Krolski, M., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2010.

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Materials

Apparatus

- 100-mL and 1000-mL glass volumetric cylinders
- Adjustable pipettors and tips
- Gastight syringe (Hamilton No. 81265)
- Analytical Balance, accuracy of 0.01 mg for analytical standards (Mettler Toledo AT-20)
- Shaker table (Eberbach Corp)
- Air Sampler (A.P.Buck, Orlando, FL, Buck-Genie VSS-12, part no. 909000)
- TurboVap (Zymark Inc., Hopkinton, MA)
- Reversed phase chromatography column (Waters, Milford, MA, XTerra MS C18, 2.5 μm, 50 mm x 2.1 mm, part no. 186000594).
- TSQ Quantum Ultra liquid chromatograph/mass spectrometer (LC-MS/MS) equipped with an electrospray interface, Surveyor HPLC pumps and autosampler, and LCQuan 2.0 data collection software (Thermo Electron Corporation)

Reagents

- Acetonitrile (ACN; HPLC Grade; Fisher No. A996-4)
- Methanol (MeOH; HPLC Grade; Fisher No. A454-4)
- Laboratory Grade Water (Optima Grade, Fisher No. W7-4)
- Aerosol OT (A-OT), 10% (w/w) (Fisher No. SA292-4)
- A-OT solution, 0.01%. Prepare by adding 1.00 mL of 10% A-OT to a 1000-mL glass or Nalgene bottle with 1000 mL of laboratory grade water. Mix well and use within 24 hours.
- Formic Acid, 88% (Fisher No. A118-500)
- Union suits (Unity Sports Apparel)
- OVS XAD-2 tubes (SKC Inc., Cat. No. 226-30-16): OSHA versatile sampling tubes (OVS-2 tubes) are 13 mm o.d. glass tubes tapered to 6 mm o.d. consisting of (order of exposure): a 13 mm diameter glass fiber filter held by a Teflon retainer ring, a 270 mg section of XAD-2 adsorbent, a foam plug, a 140-mg section of XAD-2, and a foam plug.
- Disposable 20-mL scintillation vials (Wheaton, Cat. No. 986561)
- Culture tubes, 20mm x 150mm (Fisher No. 14-961-33)
- Disposable glass Pasteur pipettes, 5 3/4" with bulbs (Fisher No. 13-678-6A)
- Disposable 60-mL vials (I-Chem, Cat. No. S236-0060) Formic Acid, 88% (Fisher No. A118-500).
- 0.1% aqueous formic acid. Prepare by diluting 1.0 mL of formic acid to 1000 mL with water and mix well.
- Gauze pads, 4 inch x 4 inch (Johnson & Johnson, SKU 8137-008525)
- Glass jars, 250 mL (Fisher No. 05-719-63)
- Glass jars, 500 mL (Wheaton Science No. W216926)
- Glass jars, 1 gallon (Fisher No. 02-911-918)

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ACS Symposium Series; American Chemical Society: Washington, DC, 2010.

- HPLC vials and caps (2-mL, National Scientific, Part Nos. C4011-5W and C4011-55)
- Octadecyl solid-phase extraction cartridges (C18 SPE), 1 mL, 0.05 g (Varian Bond Elut, Cat. No. 1210-2058).

Standards

Four target standards were used, and each had its own isotopically labeled internal standard. These are listed in table I.

Various concentrations of the standards can be made. In general, all initial and secondary standard solutions were made up in ACN, except for clothianidin's initial standard which was made up in acetone due to solubility limitations. The initial and secondary standards can be stored under frozen conditions. The final mixed calibration curve solutions were made up in water, and were stored under refrigerated conditions. Calibration solution concentrations ranged from 0.10 ng/mL to 15000 ng/mL, which related to sample concentrations of 0.005 ng/sample to 750 ng/sample for OVS tubes and 0.05 ng/sample to 7500 ng/sample for all other matrices.

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Table I. Target Standards and Isotopically Labeled Standards

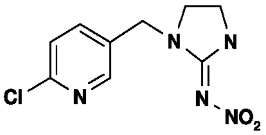
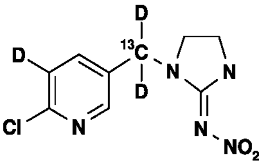
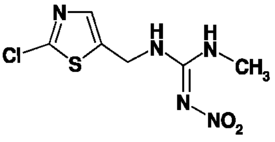
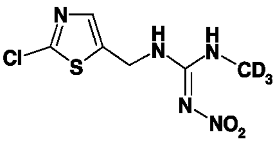
Common Name C.A. Name Molecular Wt. CAS Reg. No.	Imidacloprid 1-[(6-Chloro-3-pyridinyl) methyl]-*N*-nitro-2-imidazolidinimine 255.66 138261-41-3	
Common Name C.A. Name Molecular Wt. CAS Reg. No.	$^{13}C,d_3$-Imidacloprid 1-[(6-Chloro-3-pyridinyl-5-*d*)methyl-^{13}C-d_2]-*N*-nitro-2-imidazolidinimine 259.68 Unavailable	
Common Name C.A. Name Molecular Wt. CAS Reg. No.	Clothianidin [*C(E)*]-*N*-[(2-Chloro-5-thiazolyl)methyl]-*N′*-methyl-*N″*-nitroguanidine 249.6780 210880-92-5	
Common Name C.A. Name Molecular Wt. CAS Reg. No.	d_3-Clothianidin *N*-[(2-Chloro-5-thiazolyl) methyl]-*N′*-(methyl-d_3)-*N″*-nitroguanidine 252.6965 Unavailable	

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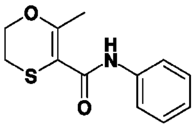
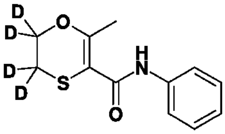
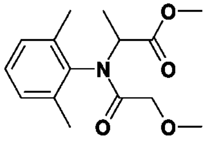
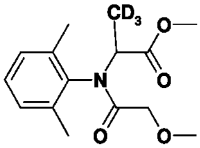
Table I. (Continued). Target Standards and Isotopically Labeled Standards

Common Name C.A. Name Molecular Wt. CAS Reg. No.	Carboxin 5,6-Dihydro-2-methyl-*N*-phenyl-1,4-oxathiin-3-carboxamide 235.3021 5234-68-4	
Common Name C.A. Name Molecular Wt. CAS Reg. No.	d_4-Carboxin Unavailable 239.3268 Unavailable	
Common Name C.A. Name Molecular Wt. CAS Reg. No.	Metalaxyl Methyl *N*-(2,6-dimethylphenyl)-*N*-(2-methoxyacetyl)alanine 279.3315 57837-19-1	
Common Name C.A. Name Molecular Wt. CAS Reg. No.	d_3-Metalaxyl $(^2H_3)$Methyl *N*-(2,6-dimethylphenyl)-*N*-(methoxyacetyl)alaninate 282.3500 Unavailable	

Extraction Procedures

Dosimeters

Dosimeters were worn under clothes to approximate dermal exposure during the working day. After use, they were cut and separated into 6 segments: combined lower arms, combined upper arms, combined lower legs, combined upper legs, front torso, and back torso. These individual segments were wrapped in aluminum foil for shipment. Control segments were fortified with a mixed solution of all four compounds of interest (field fortification) prior to shipment.

Once received at the analytical lab, the extraction procedure was:

- Place a single piece and it’s foil wrapping into a 1 gallon jar.
- Add 2 liters of methanol to jar.
- Dose with an appropriate volume of mixed internal standard.
- Shake sample for 15 minutes.
- Condition a C-18 cartridge with methanol.
- Load cartridge with 5 mL of sample extract.
- Collect the eluent into culture tube.
- Evaporate the eluent to dryness.
- Add ~1 mL water and mix well.
- Transfer the sample to an autosampler vial for analysis.

Note: the C-18 was used to remove any salt present in the extract.

Face and Neck Wipes

Workers wipe off their face and neck throughout the day with gauze pads moistened with the 0.01% Aerosol OT (A-OT) solution. These wipes were collected on foil and shipped. Control pads were fortified with a mixed solution of all four compounds of interest (field fortification) prior to shipment.

Once received at the analytical lab, the extraction procedure was:

- Place face wipes and it’s foil wrapping into a 500 mL container.
- Add 300 mL methanol to the container
- Fortify with an appropriate volume of mixed internal standard.
- Shake sample for 15 minutes.
- Condition a C-18 cartridge with methanol.
- Load cartridge with cartridge volume of sample extract, and collect eluent into a culture tube.
- Evaporate the sample to dryness.
- Add ~1 mL water and mix well.
- Transfer the sample to an autosampler vial for analysis.

Note: the C-18 was used to remove salt and A-OT present in the extract.

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Hand Washes

Workers wash hands several times during the day with the 0.01% A-OT solution. These washes were collected in basins but transferred to glass bottles. Fresh, unused A-OT solutions were fortified with a mixed solution of all four compounds of interest (field fortification).

Part of the extraction was conducted at the seed treatment facility prior to shipment:

- Fortify samples with an appropriate volume of mixed internal standard.
- Prepare 2 C-18 cartridges for each wash.
- Load a cartridge volume (CV) of hand wash solution onto each cartridge.
- Wash cartridges with a CV of water.
- Vacuum dry the cartridges for shipment.

Once received at the analytical lab, the extraction procedure continued:

- Place cartridge into a culture tube.
- Elute cartridge with methanol.
- Evaporate to dryness.
- Add ~1 mL of water and mix well.
- Transfer the sample to an autosampler vial for analysis.

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OVS Tubes

Inhalation exposure was estimated using a personal air pump attached to an OSHA Versatile Sampler (OVS) tube placed in the breathing zone of the subject. Fortification samples were OVS tubes that were fortified on the upper absorbent layer and had air pumped through them for several hours prior to shipment.

Once received at the analytical lab, the extraction procedure was:

- Score the small (lower) tube section and remove bottom glass.
- Place lower foam plug and lower sorbent sampling section into a 20 mL vial. Add 10 mL of methanol to the vial.
- Place remaining contents (including the upper sorbent section and the holding ring) into a separate 20 mL vial. Rinse the glass OVS tube with 10 mL of methanol, and capture that 10 mL in the upper sorbent vial.
- Fortify with an appropriate volume of mixed internal standard.
- Shake for 30 minutes.
- Place a 0.25 mL aliquot of each extract into a separate autosampler vial.
- Add 0.75 mL of water to each vial and cap for analysis.

Instrumental Procedures

High Performance Liquid Chromatography

High performance liquid chromatography (HPLC) separation was performed with a Waters XTerra MS C18, 50 mm x 2.1 mm column, using 0.1% aqueous formic acid and methanol as mobile phases on a Thermo Finnigan Surveyor HPLC. The column was heated to 40 °C. The gradient and flow is described in Table II.

The standard concentrations for the linearity curve ranged from 0.1 ng/mL to 15000 ng/mL. The injection volumes were varied between 3 µL and 20 µL to avoid detector saturation when injecting high level standards or samples and to avoid poor signal when injecting the low level standards or samples. Therefore, the high level standards and fortifications were injected at 3 or 5 µL while the low concentration standards and fortifications were injected at 15 -20 µL.

Mass Spectrometry

The HPLC interfaced to a ThermoFinnigan Quantum Ultra tandem mass spectrometer for analyte detection. Quantitation of imidacloprid, clothianidin, carboxin and metalaxyl residues was conducted on all samples using selected reaction monitoring liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) in the positive ion mode, observing a selected transition from the molecular ion to a single product ion for each analyte. See Table III for additional information on the mass spectrometer conditions. All components were analyzed within the same instrumental run for a single sample.

Table II. HPLC conditions

Time (min)	*Flow (ul/min)*	*% Aqueous*	*% Methanol*
0.00	300	80	20
0.50	300	80	20
4.50	300	40	60
5.50	300	40	60
5.51	300	80	20
6.00	600	80	20
8.50	600	80	20

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Table III. Mass Spectrometer Conditions

Component:	*Imidacloprid*	*Clothianidin*	*Carboxin*	*Metalaxyl*
Retention Time(min):	2.30	2.30	5.40	6.00
Native Molecular Ion:	255.9	249.9	236	280
Native Product Ion	209.0	169.0	143.0	220.1
IS Molecular Ion:	259.9	252.9	240.0	283.0
IS Product Ion:	213.0	172.0	146.9	220.1
Spray Voltage:	4800	4800	4600	4600
Sheath Gas Pressure:	70	70	60	60
Aux Gas Pressure:	20	20	30	30
Ion Sweep Gas Pressure:	5.0	5.0	6.0	6.0
API Temp:	310	310	310	310
Resolution for Q1MS:	0.7	0.7	0.7	0.7
Skimmer offset(v):	9	9	9	9
Collision Energy(v):	17	14	17	15
Q2 Collision Gas (mT):	0.9	0.9	0.9	0.9
Resolution for Q3MS:	0.7	0.7	0.7	0.7

Linear regression was used to generate a calibration curve for each analyte. The concentration of analyte was determined from a calibration curve generated during sample analysis using the following equation:

$$\text{ng/sample found} = [([(N/IS)-i])/m] * D$$

Where:
N = the peak area for the native analyte
IS = the peak area for the internal standard
i = intercept of calibration line
m = slope of the standard calibration curve
D = the dilution factor (if applicable)

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Results and Discussion

Overview of Samples Received

Samples from 66 workers were collected at the seed treatment facilities. Each MU yielded 6 dosimeter pieces, 2 OVS tube sections, 3-6 hand washes, and 1 face and neck wipe sample. This gave a total of 865 samples from the workers. In addition, each site generated field fortifications for each sample type (dosimeter, OVS tube, hand wash, face and neck wipe). Finally, each sample type required method validation at the LOQ and at 10 times the LOQ. In total, 1060 lab samples were created for analysis. These were received, extracted, and analyzed within the course of 60 working days.

Analysis Techniques

With four separate components of interest, the 1060 samples received meant that there were 4240 data points to be acquired over the course of the study. Several techniques were employed to allow the laboratory analysis to be completed in 60 working days.

The initial hand wash preparation (loading an aliquot of the sample onto a C-18 cartridge prior to shipment) was done at the test facility. There were several advantages to doing this. Primarily, there was significant sample size reduction, since every 500 mL sample was reduced to 2 small plastic columns. Therefore, the resulting samples were smaller and lighter, resulting in a more affordable shipment. Plus, the cartridges were sturdier, and there were no lost samples due to broken glass bottles. Finally, due to the smaller size, the received samples were easier to store and extract in the laboratory.

The use of isotopically labeled internal standards also aided in the analysis of samples. This is due to the fact than instead of measuring a compound’s area captured on the mass spectrometer, the ratio between the native compound and its internal standard response was measured. If a clean up method did incur some loss of the native component, the internal standard will be lost at a proportional rate, and the ratio would remain the same. Therefore, the use of internal standards increased preparation speed by eliminating specific volume measurements and repeated washes when transferring samples from one container to another. Also, it allowed variable injection sizes, compensating for detection or saturation limits of the mass spectrometer in a study with a large range of standard concentrations.

Due the large number of samples and individual data points, it was essential that a single injection be used to measure all components in a sample. This minimized the amount of time that the instrument was used and allowed more rapid review of the data. See Figure 1 for an example of the chromatograms attained during the course of the study.

Instrument Detector Response – Range and Linearity

For dosimeter garments, face and neck wipes, and hand washes, the response of the LC/MS/MS to all components was linear over a range of 0.05 ng/sample to 7500 ng/sample. For OVS tubes, the response of the LC/MS/MS to clothianidin, carboxin, and metylaxyl was linear over a range of 0.005 ng/sample to 750 ng/sample. The coefficients of determination of the linearity curves were all ≥ 0.99.

Method Validation and Limit of Quantitation

The method was successfully validated by analysis of untreated control (UTC) samples fortified with imidacloprid, clothianidin, carboxin, and metylaxyl. The method limit of quantitation (LOQ) was the lowest fortification level at which acceptable recovery was achieved. The established LOQ for face wipes, hand washes, and dosimeter garments was 0.10 ug/sample. The LOQ for OVS tubes was 0.01 ug/sample.

Limit of Detection

The limit of detection (LOD) was the lowest analyte concentration giving a measurement statistically different from that of a blank UTC sample. The individual LOD's for imidacloprid, clothianidin, carboxin, and metylaxyl in each matrix were calculated by multiplying SD, the standard deviation of the analyte recovery measurements at the LOQ, by $t_{0.99}$, the appropriate one-tailed Student's t statistic, and adding this value to the average analyte residue found in the blank UTC samples of that matrix. See Table IV for the LOD values for each compound in each sample type.

Field Fortification Recoveries

Analysis of blank untreated control (UTC) samples fortified in the field with mixtures of the monitored test chemicals was performed concurrently with sample analysis to verify sample integrity and to verify method performance. Measured residues in each matrix were adjusted based on the recovery of field-fortified samples analyzed concurrently with each sample set. A summary of recovery data for all compounds and matrices is given in Table IV.

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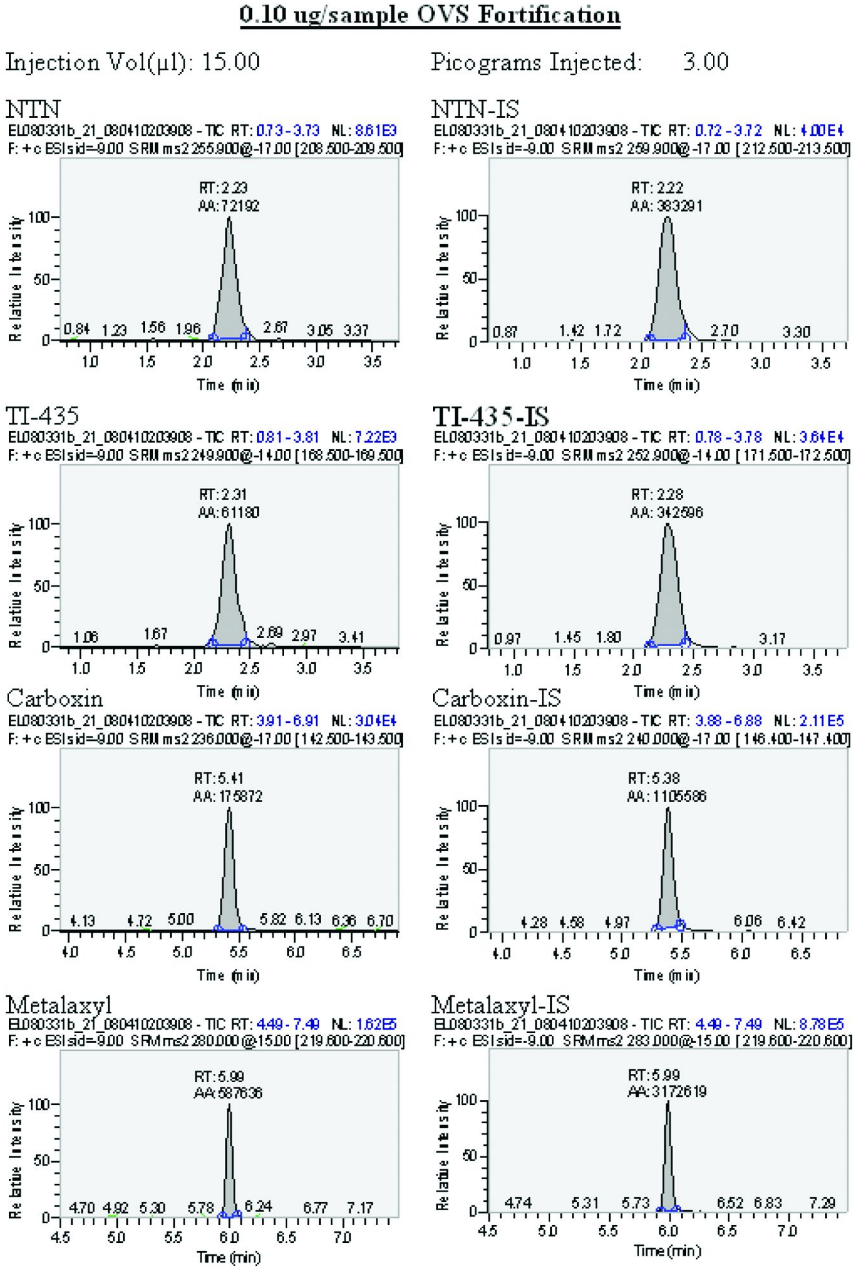

Figure 1. Low Fortification of OVS tube

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Table IV. Recovery and LOD results

Compound	*Sample Type*	*LOD (ug/ sample)*	*Spike Levels (ug/sample)*	*Mean % Recovery*	*Stan. % Dev.*
Imidacloprid	Dosimeters	0.025	0.1 - 5000	98%	12%
	Face and Neck Wipes	0.031	0.1 - 2500	93%	12%
	Hand Washes	0.034	0.1 - 5000	91%	17%
	OVS Tubes	0.0032	0.01 - 600	87%	8%
Clothianidin	Dosimeters	0.075	0.1 - 5000	94%	16%
	Face and Neck Wipes	0.024	0.1 – 5000	98%	14%
	Hand Washes	0.007	0.1 - 5000	87%	15%
	OVS Tubes	0.0024	0.01 - 600	84%	8%
Metalaxyl	Dosimeters	0.026	0.1 - 5000	106%	6%
	Face and Neck Wipes	0.054	0.1 - 2500	107%	11%
	Hand Washes	0.032	0.1 - 5000	106%	9%
	OVS Tubes	0.0042	0.01 - 600	106%	12%
Carboxin	Dosimeters	0.036	0.1 - 5000	96%	17%
	Face and Neck Wipes	0.040	0.1 - 2500	95%	19%
	Hand Washes	0.026	0.1 - 5000	96%	18%
	OVS Tubes	0.0011	0.01 - 100.0	59%	26%

Conclusion

This type of study generated a large number of samples in a very short period of time. Developing analytical methods with similar techniques allowed rapid sample throughput and quick adaptation of the lab from one sample type to another. The use of internal standards aided this as well, as did the analysis of all components within one instrument injection.

The method was successfully validated and verified over a large range of standard concentrations for all four components. Overall, the extraction, clean up and analysis of the 1060 samples (validation & field samples) received was completed in under 60 working days.

In Non-Dietary Human Exposure and Risk Assessment; Krolski, M., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2010.

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